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ORIGINAL ARTICLES

A NOTE ON THE BLOOD CHLORIDES IN MERCURIC CHLORIDE NEPHROSIS*

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COMPARATIVE studies of the composition of the blood and urine in a case of mercurial poisoning were reported in 1915 by Myers and Fine[1] who observed that the accumulation of nonprotein and urea nitrogen, of the uric acid and of the creatinine in the blood was coincident with a diminished output of these compounds in the urine. After 5 days of anuria, the nonprotein nitrogen of the blood had risen to 338, the urea nitrogen to 240, the uric acid to 15, and the creatinine to 33 mg. per 100 c.c. After decapsulation of the kidneys a temporary improvement of renal function was noted with a decrease of the concentration of these nitrogenous constituents in the blood, and an increased output in the urine. A subsequent decline of kidney activity, however, resulted in death. The authors emphasized the diagnostic and prognostic significance of the chemical analysis of the blood in mercuric chloride nephrosis, and in chronic nephritis. A review of the literature since that time reveals abundant data upon changes in the concentration of the nitrogenous components of the blood following mercuric chloride poisoning but comparatively little attention has been accorded other equally important constituents. In a nonfatal and less severe case, described by Cohen and Bernhard,[2] the clinical improvement was preceded by a marked decrease in the nonprotein and urea nitrogen, and of the creatinine of the blood after the introduction of large quantities of alkaline fluid by mouth, rectum, and under the skin. Similar observations are recorded by Rosenbloom,[3] Underhill[4] and Campbell.[5]

It is significant that in mercuric chloride nephrosis despite the severe impairment of renal function indicated by the marked retention of the nitrogenous waste products, edema is generally absent. In other types of nephrosis,

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however, an anasarca usually develops consequent to an impairment of chloride excretion. Campbell drew attention to this fact in his studies on a fatal case of mercuric chloride poisoning. Here the chlorides of the blood plasma had dropped within 8 days from 0.666 to 0.521 per cent, whereas the nonprotein nitrogen rose from 170 to 319 mg. per 100 c.c., and the sugar from 0.20 to 0.46 per cent. That the retention of the nonprotein nitrogenous components of the blood in this type of kidney injury is not accompanied by an increase in the blood chlorides is further evident from the work of Lewis and Rivers.[6] It was noted by them that in a case of bichloride of mercury poisoning, after the ingestion of large quantities of fluid, the plasma chlorides reached their lowest level (0.385 per cent) when the nonprotein nitrogen was at its maximum concentration (178 mg. per 100 c.c.). After 14 days of treatment leading to recovery the nonprotein nitrogen had been decreased to 22 mg. but the plasma chlorides had returned to 0.595 per cent. In like manner, Woods[7] has reported a low concentration of the chlorides of the whole blood (0.342 per cent) coincident with a pronounced retention of the nitrogenous waste products in one case of bichloride poisoning, and in several cases of chronic nephritis. Although a high blood chloride was usually found in the cases of severe nephritis studied by Myers,[8] in one instance a restriction of the chlorides of the diet and a large fluid intake produced a drop in the concentration of the chlorides of the whole blood from 0.594 to 0.394 per cent, but during the same period the nitrogenous waste products were markedly increased.

MacNider[9] has emphasized the influence of the disturbance of the acid-base equilibrium of the blood in the production of the kidney injury in mercurial poisoning. He noted that in the animals surviving the gastroenteritis following poisoning with mercuric chloride the injury to the kidney was constantly associated with the development of an acid intoxication. The extent of the impairment of renal function bore no relation to the elimination of the mercury in the urine, and was not due to the action of the metal upon the kidney tissue. However, the progress of the kidney insufficiency kept pace with the drop in the alkali reserve of the blood and the excretion of ketone bodies. A restoration of the normal acid-base equilibrium of the blood was accompanied by a return to normal of the functional capacity of the kidneys.

The object of the present communication is to present the results of observations on two nonfatal cases of mercurial poisoning. It is believed that these results, though not entirely new, may be of interest in view of the points previously discussed. These studies comprise analysis of the blood, specimens of which were obtained at frequent intervals, for urea nitrogen, uric acid, creatinine, chlorides (of the whole blood), sugar, and the carbon dioxide combining power. The methods of analysis described by Myers[10] were employed in all instances. Daily routine examinations of the urine were made. The total fluid intake and output were measured each day, and the stomach washings and specimens of urine were examined for mercury by the method of Vogel and Lee[11] until these tests proved the absence of the metal. Significant clinical changes are also noted.

TABLE I

CHEMICAL BLOOD FINDINGS IN BICHLORIDE POISONING WITH SPECIAL REFERENCE TO THE CHLORIDES

CASE	DATE 1921	CHLORIDES AS NaCl	URIC ACID	UREA N	CREATININE	CO_2 COMBINING POWER
		per cent	mg.	to	100 c.c.	c.c. to 100
1.—R. S., female, aged 19, 15 grains $HgCl_2$ taken	May 9	0.495		22		36
	" 11	0.338	8.3	90	10.7	
	" 13	0.388		88	9.1	30
	" 15	0.114		98	12.0	27
	" 17	0.207		73	12.0	
	" 19	0.250	8.0	75	9.3	
	" 21	0.363	2.8	91	7.8	81
	" 27	0.500	1.0	18	4.4	61
	June 6	0.525	1.9	13	2.2	
2.—E. F., female, aged 20, 15 grains $HgCl_2$ taken	May 17	0.382	9.6	146	14.5	36
	" 23	0.410	10.9	188	15.8	25
	June 11	0.563	1.5	11	2.2	

Case 1.—Admitted to Lincoln Hospital evening of May 7. Four to five liters of alkaline fluid introduced as gastric lavage, colonic irrigations and enemas; and hot packs given from May 7 to 27. In addition to this, 1000 c.c. of 0.7 per cent NaCl was introduced hypodermically daily from May 16 to 25. No anuria and no edema. Phenolsulphonephthalein excreted in traces only until May 25; at this time output was 25 per cent, and rose to 45 per cent on June 13. Diet fluid and salt-free. Clinical improvement noted about May 20. Discharged from hospital cured June 19.

Case 2.—Admitted to Harlem Hospital May 11. Gastric lavage with alkaline fluids for 4 days. One thousand c.c. Fischer's solution given intravenously daily from May 11 to 14. High colonic irrigations, large quantities of fluids by mouth, and hot packs given daily until June 11. Diet fluid and salt-free. Clinical improvement noted about June 8. Discharged from hospital cured June 15.

DISCUSSION

Both cases present evidence of severe nitrogen retention, which is more marked in Case 2 than in Case 1. It will be noted in Case 1 that the retention of urea nitrogen began about 36 hours after the ingestion of the mercuric chloride. In the second case no specimens of blood were obtained until 72 hours after the administration of the poison, and at this time the nonprotein nitrogenous constituents of the blood had already reached a very high level. Within 8 days the urea nitrogen in Case 1 had been increased steadily to 98, and the creatinine to 12 mg. per 100 c.c. Following this, a gradual clinical improvement was noted, and this clinical improvement parallels the decrease in the uric acid, urea nitrogen and creatinine of the blood. It is worthy of note that the decrease in the uric acid precedes the drop of both the urea nitrogen and of the creatinine. The same may be said to be true of Case 2. In this particular, our findings are in close accord with the observations of Cohen and Bernhard, Rosenbloom, Underhill, and Campbell.. These two patients, and as well the cases described by the authors mentioned, received large quantities of fluid by mouth, rectum, and hypodermically, and in Case 2 intravenously. The fatal case described by Myers and Fine manifested a more marked retention of the nitrogenous waste products than any of patients referred to. In this case, however, there was no attempt to increase the fluid intake, which fact may account for the greater retention of nitrogen.

Since Myers and Short[12] have pointed out that, as an index of any significant change in the concentration of the blood chlorides, a determination of the chlorides of the whole blood is more trustworthy than a determination of the chlorides of the plasma, our studies have been confined to the chlorides of the whole blood. These authors have placed the normal chlorides of whole blood between 0.45-0.52 per cent. A remarkable decrease in the blood chlorides from 0.495 to 0.114 per cent is noted in Case 1 within the first 8 days of treatment, but the nitrogenous constituents however, rose to their maximum concentration. During this period the patient was receiving per day a total of 4-5 liters of alkaline fluids containing no chlorides. For the succeeding 8 days a hypodermoclysis of 1000 c.c. of 0.7 per cent sodium chloride solution was given daily. There follows a gradual rise in the concentration of blood chlorides to 0.525 per cent. Simultaneous with this increase in the chlorides, there is a diminution of the nitrogenous constituents of the blood, keeping pace with the observed clinical improvement. In like manner in Case 2 with a pronounced retention of nitrogen, low figures were obtained for the blood chlorides. This patient, however, at no time manifested as marked a decrease in chlorides as Case 1, which may be due to the fact that Case 2 had received for the first 3 days of treatment, 1000 c.c. of Fischer's solution intravenously. Here again, with a restoration of the normal values for the nitrogenous waste products, the blood chlorides return to an unusually high level, 0.563 per cent. The work of Lewis and Rivers demonstrates that the reduction of the blood chlorides in mercurial poisoning cannot be explained by a flushing out of the salts in the urine after the ingestion of large quantities of fluid. On the contrary they found low blood chlorides coincident with a diminished excretion of salt, and as the chlorides of the blood rose there was a proportionate increase in their excretion in the urine. Myers and Fine also observed a subnormal excretion of chlorides associated with comparatively low blood chlorides. Furthermore, there was not sufficient variation in the hemoglobin, or in the enumeration of the blood cells to warrant attributing the reduction in the chlorides to a dilution of the blood.

McLean[13] has reported somewhat analogous findings for the chlorides of the blood and urine in lobar pneumonia. During the active stage of this disease he finds the failure of the kidney to excrete chlorides to be associated with a subnormal concentration of chlorides in the blood plasma. At the crisis the plasma chlorides rose abruptly and excretion began and continued in proportion to the concentration in the plasma. It would appear, then, that in mercuric chloride nephrosis, as in lobar pneumonia, the diminished excretion of chlorides must be ascribed to a decrease of the blood chlorides below the threshold point, and with a rise of the chlorides the amount excreted in the urine proportionately increases. The mechanism of chloride retention with a simultaneous decrease of the chloride concentration in the blood in mercuric chloride nephrosis is at the present time an obscure point.

At the time of the greatest impairment of kidney function, there was observed a marked decrease in the carbon dioxide combining power of the blood plasma. In Case 1 the carbon dioxide combining power dropped to 27,

and in Case 2 to 25 volumes per cent. A rise in alkali reserve of the blood plasma in the first instance followed the use of alkaline fluids, in gastric lavage, colonic irrigations and enemas. This rise accompanied the improvement of renal function. These findings are of interest in view of MacNider's contention, but whether the restoration of the normal functional capacity of kidneys is to be explained by the protecting action of the alkalies cannot be decided by our data.

It is also worthy of note that in both cases no evidence of edema was presented at any time. The volumes of urine excreted in 24 hours were moderately increased, with specific gravities ranging from 1008 to 1022. In both instances, occasionally sugar was detected in the urine with Benedict's qualitative reagent although the blood sugar did not rise above 0.114 per cent. Glycosuria is not an unusual finding in certain types of kidney disease particularly after poisoning by metallic salts. Myers and Kast[14] have reported glycosuria in 3 cases of chronic nephritis with but slight nitrogen retention, but with edema and constant proteinuria. In one instance there was a normal blood sugar, and in the other two but slight hyperglycemias. The glycosuria in the latter was found to be independent of the hyperglycemia. Cohen and Bernhard, likewise, noted a glycosuria of 1.5 per cent with a hypoglycemia. An examination of the ocular fundi revealed nothing abnormal.

SUMMARY

Two nonfatal cases of mercuric chloride poisoning are reported. As the impairment of renal function due to kidney injury progressed, a diminution of concentration of the chlorides of the whole blood was noted. A return to normal of the functional capacity of the kidneys was accompanied by an increase in the blood chlorides.

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CLINICAL DIAGNOSIS BY THE AID OF VISCOSIMETRY OF THE BLOOD AND THE SERUM WITH SPECIAL REFERENCE TO THE VISCOSIMETER OF W. R. HESS

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THE determination of the viscosity of the blood in the study of hemodynamics (physiology of the circulation) and in general diagnosis has not received the attention of clinicians because of the lack of a practical manner of its determination and the difficulty in the interpretation of results. During the last twenty years approximately 200 papers have been published on the subject, most of them after the introduction in 1906 of the viscosimeter of Hess,[20] the only apparatus which fulfills all the requirements for a practical and accurate test. But contradictions and uncertainty concerning the application of the test prevailed until recently a practical clinical basis was established for it by Naegeli.

English and American investigators have contributed about twenty articles on the viscosity of the blood; in this country Burton-Opitz has been one of the most industrious pioneers. His valuable contribution is too purely physiologic in nature for clinical application, although his results merit an attentive study, especially since the apparatus of Hess replaces his more complicated technic. His conclusion may be quoted here as it was presented to the American medical profession in 1911 to emphasize the value of a broader consideration of the viscosity factor in circulation: "The viscosity represents an independent dynamic power which, although slight normally, will become overwhelmingly great, if other factors favor its development."[15] McCaskey, in 1908, presented a very simple and effective viscosimeter, but the duration of the determination extends into the phase of coagulation. The best English review on the manifold questions related to the viscosity of blood was published in 1911 by Allbutt who gave a broad conception of the relation of viscosity to the physiology of circulation, discussed all the known facts pointing to the difficulty of interpretation, and predicted a more general recognition of its significance in the future. Recently Langstroth studied the viscosity of the blood after its withdrawal from the body. These studies are of theoretic value only, corroborating old findings and presenting many errors because the thorough previous investigations are not considered carefully enough.

The discussion of theoretic problems concerning the viscosity of the blood will be limited in this review on account of the space which it seems advisable to give to more practical details, in order that any practitioner or clinician may have a basis for the application of viscosimetry, and may successfully carry it out in his laboratory and at the bedside.

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VISCOSIMETER OF HESS

The viscosimeter of Hess is not only the simplest, but also the most reliable apparatus, especially for high values, since it eliminates many technical errors. Hess devoted several critical studies to his own apparatus, and was able to give clear scientific proof of the principles involved in its mechanism. Independently Kagan, of Sahli's Clinic, in an elaborate criticism of the different viscosimeters presented a satisfactory answer to all the possible objections to Hess's instrument.

The apparatus is based on the law of Poiseuille,* according to which fluids, at equal temperature and equal pressure, passing through capillary tubes of equal caliber, vary in their rate of flow in direct proportion to their internal

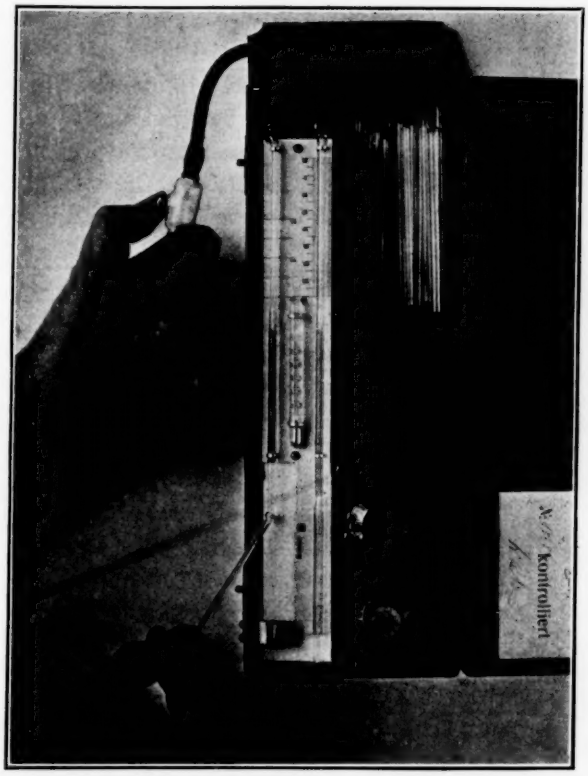

Fig. 1.—Viscosimeter of W. R. Hess. The picture illustrates the handling of the apparatus the moment the blood drop is drawn in.

friction, that is, to their viscosity. The law can be applied only when liquids are driven through the capillaries by a certain pressure. This pressure should be moderate so as to obtain a gentle flow of the liquid, but it must be greater than the force of gravity. The latter is employed in many other instruments and its incompatibility with the law of Poiseuille is the chief objection against the well-known apparatus of Determann (Fig. 1).

By the aid of a strong rubber bulb, blood and distilled water are drawn simultaneously under the same pressure through two capillaries. If the cap-

*Poiseuille was the first to study the problem of viscosity and published his fundamental law in 1847.

illaries are of equal length and lumen, and if the temperature, pressure, and time are the same for both capillaries, the flow through volume of two liquids is inversely proportional to the viscosity. The volume is measured by a graduated glass tube, and the volume of water taken as the standard. The flow through volume of any fluid more viscous than water will be smaller than that of water, or the flow through volume of water will be just so much larger as the tested fluid is more viscous. If blood is sucked from 0 to 1 the water column reaches a point in the graduated tube which indicates directly how much farther the water was flowing under the same conditions. At this point a reading is taken. This reading is exactly the viscosity of the blood, compared with water, and is therefore relative.

The viscosity of a fluid is expressed in viscosimetric units of distilled water at a temperature of 20° C. Within certain limits it is not necessary to consider the temperature, although this may easily be done. The temperature recorded by the thermometer which is placed between the capillaries, should be between 17°C. and 23°C. Tests made at these temperatures need no correction because the error will not exceed ± 3 per cent when 20° C. is taken for the standard. Any deviation from these limits is corrected by adding for every degree

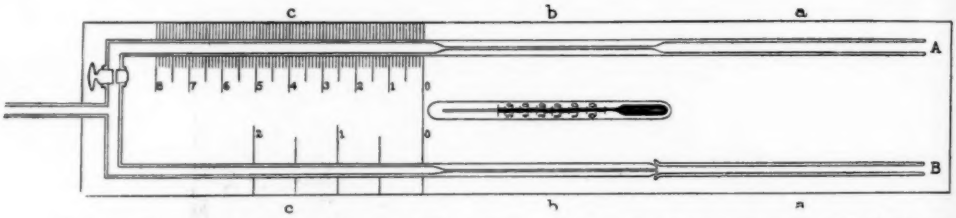

Fig. 2.—Diagram of the viscosimeter of Hess. The flow through volumes of water and blood are measured in the respective tubes (*c.c.*). The two capillaries (*b.b.*) are of exactly the same size, but the water tube for measurement is slightly larger than the glass tube as distinctly shown in the diagram. This fact explains why the units of the water tube are smaller than those of the blood tube, but it must be understood that the volumes are exactly the same.

above 20° C., 0.8 per cent of the reading, and by subtracting 0.8 per cent for every degree below 20° C. For laboratory research Hess has devised a larger model with a water cylinder, which makes it possible to carry out series of determinations at constant temperatures.

DESCRIPTION OF PARTS

I. The viscosimeter is composed of the following parts (Figs. 1 and 2).

1. Box, serving as a basis for the test capillaries, and containing all the necessary ingredients for a complete test.
2. Two glass tubes, *A* for distilled water, *B* for blood, united at one end by an u-shaped tube with a common outlet.

Tube *A*.

a. Recipient for distilled water.

b. Test capillary.

c. Tube for measurement, graduated in viscosimetric units.

By a stop-cock at the end of tube *c* the water system can be separated from the pressure system.

Tube *B*.

a. A removable receptor for blood.

b. Test capillary.

c. Tube for measurement (empiric scale).

3. Rubber tube, connecting the common outlet of the U-shaped tube with the glass valve.
4. Nonautomatic glass valve: enlarged glass tube with lateral hole.
5. Rubber bulb.
6. Set of small glass tubes, used as blood receptors, and several larger cleaning tubes.
7. Bottle with ammonia.
8. Bottle with distilled water.

II. Handling the apparatus.

A. Establishing the movements:

a. Suction: first compression of bulb, then closure of valve hole with thumb. Release of bulb should be slow.

b. Pressure: closure of valve hole first, then compression of the bulb.

c. Sudden release of suction or pressure to stop the flow of the fluid columns: the thumb is lifted from the hole of the valve.

B. Filling the glass tubes:

1. Water Tube *A*.

a. Cleaning tube is filled with pure distilled water.

b. The stopcock is opened and suction produced.

c. The cleaning tube is adjusted to opening of Tube *A*, so that the water meniscus touches the opening first.

d. The water is drawn in until column reaches mark "O," where meniscus is stopped by releasing thumb from valve.

e. Closure of stopcock.

2. Blood Tube *B*.

a. Blood receptor is filled with a fresh drop of blood, which must enter the tube spontaneously (any hesitation indicates that the recipient is not clean).

b. Suction is produced first, then receptor adjusted to the opening of blood Tube *B*.

c. The blood column is drawn to mark "O", and suction released.

C. Completing the test:

a. Menisci are controlled, that both touch mark "O".

b. Stopcock is opened and suction produced.

c. Stopping of suction when blood column has reached mark "1" (serum is drawn to mark "2", very thick blood to mark "0,5").

d. Reading of position of water meniscus gives directly the relative viscosity.

e. Both columns are forced back to mark "O", the stopcock closed and the blood expelled as quickly as possible.

D. Cleaning the instrument:

One should clean the blood capillary immediately after use by drawing ammonia back and forth. If the apparatus is not used, pure ammonia should be left in the capillary.

The capillaries should be as clean as possible. In cases of erroneous readings there is a doubt in the cleanliness of the instrument and fuming nitric acid should be used first, then water, alcohol, and ether.

When emptying the capillaries, wet cotton should always be used to catch the liquid and air bubbles at the opening.

The blood receptors have to be cleaned at once by removing the blood with water. They are left in fuming nitric acid for twelve to twenty-four hours, after which they are rinsed with distilled water and dried on a wire gauze over a Bunsen burner.

To escape the danger of having the delicate capillaries obstructed by coagulated blood, water, sugar-solutions, serum, alcohol, etc., should be used first, until it is possible to run the test automatically.

III. Preparing the patient:

The patient is prepared by having his hands submerged in hot water (42° C.) for five minutes, asking him to rub them together constantly. This results in a

hyperemia of the capillary net of the hand. The blood, obtained by a small incision in the end of the finger is sufficiently arterialized to give a constant value. Venous stasis increases the viscosity of the blood in consequence of an increase in the size of the red cells through the influence of carbon dioxid. Such blood shows great variation in viscosity.

VALUE OF THE DETERMINATION OF VISCOSITY

The viscosity is a very definite property of the blood. It is a body-constant like the temperature, and, in the same individual, remains the same, including the daily variation, over long periods. A deviation from the usual rate takes place only after drastic procedures or after a permanent change in diet. There is still a doubt whether the viscosity has a specific purpose, as, for instance, to prevent the inhibition of the tissues by the blood fluid, or whether the viscosity is the unavoidable result of certain blood ingredients. The lower limit of their concentration is indicated by the demand for supply of the body cells. The blood must carry a certain load of cellular elements and high viscous protein. The resulting viscous fluidity increases the work of the heart to many times what it would be if the blood had the viscosity of water. The viscosity in relation to heart work therefore becomes a very important factor in circulatory and cardiac disturbances. Hess believes that an increase in the number of red cells is followed by an increase in concentration of the dissolved protein. This is an additional factor of no less importance, which, however, is only mentioned in this connection; for mathematic formulation see Hess' work.

The viscosity is also a very complex property of the blood, and may be considered to represent one side of an equation, the other side of which is formed by a series of variable factors. The influence of these factors is not merely a summation, but is rapidly intensified with an increase in concentration. These factors are easily determined and the viscosity represents then a very valuable and exquisite check on the accuracy of the other values. Every deviation of the expected η-value demands an explanation that will lead to the discovery of an error or to a cause not yet considered. Furthermore, the viscosity represents an especially fit test for rapid controls of the blood (routine tests). Naegeli states that the determination of the viscosity must be part of an accurate blood test, but that it is of importance to have the complex value of η analyzed.

DISCUSSION OF THE VARIOUS FACTORS OF THE VISCOSITY OF THE BLOOD

Practically the most important factors which contribute to the complex value of the viscosity of the blood are the serum and the cellular elements. Naegeli enumerates some other factors, but their influence is almost negligible under the most frequently studied conditions. The influence of carbon dioxide is eliminated by the hand-bath which avoids congested blood for the determination. Nevertheless we find a specific influence of carbon dioxide in general cyanosis, which can be recognized when the precaution of inducing hyperemia is routinely applied.

Experiments which I carried out in 1919 in the physiologic laboratory of Hess have clearly demonstrated the prevalent influence of the dissolved protein in the serum. The delicacy of the method was proved by the fact that differences

of the protein were readily revealed, and that it was possible to differentiate protein by a so-called viscosimetric index. Other experiments specified the influence of the volume of red blood cells on the viscosity of the blood, at least viscosimetry was shown to give an exact determination of the cell volume (Ulmer-Bircher method).

VISCOSITY OF THE SERUM η_1 (PLASMA η_2)

1. In normal conditions the viscosity of the serum ranges from 1.7 to 2.0 (plasma from 0.2 to 0.3 higher).

2. In pathologic conditions the lowest value of the serum found by Naegeli was 1.45 in pernicious anemia, and the highest 2.3 in plethora.

The influence of salt dissolved in the serum is negligible within certain limits; preponderant is the dissolved protein, and viscosimetry is therefore an approximate method of estimating the protein content of the serum.

TABLE I (Naegeli)

PROTEIN CONTENT OF THE SERUM PER CENT	VISCOSITY IN VISCOSIMETRIC UNITS
5.0	1.43
5.5	1.46
6.0	1.57
6.5	1.56
7.0	1.61
7.5	1.67
8.0	1.72
8.5	1.78
9.0	1.84
9.5	1.90

CELLULAR ELEMENTS

Red Cells.—The viscosity is directly related to the number of red blood cells or, more correctly, to the total volume of red blood cells. A practical method of estimating the exact total volume of the red blood cells and the volume of each cell is discussed in Section III.

The volume of the cells determines the viscosity of the blood. This is a very important fact for the differential diagnosis of pernicious anemia and secondary anemia. While in pernicious anemia the size of the red cells does not permit the viscosity to decrease in proportion to the low count, in secondary anemia the viscosity is very low, proving that there are no cells of increased size. This interesting fact shows, too, that the megalocytes in pernicious anemia must be much more prevalent than has been believed.

White Cells.—These cells do not influence the viscosity in normal blood owing to their small number, but they have a marked influence in pathologic conditions in which the definite relation of the cell volume to the viscosity permits a differential diagnosis, example of which is shown in Table II.

TABLE II (Naegeli)

	MYELOID LEUKEMIA	LYMPHATIC LEUKEMIA
Red cells	4,540,000	5,650,000
Hemoglobin	90.0 per cent	110.0 per cent
Color index	1.0	1.0
Leucocytes	396,000	44,240
Viscosity	7.3	4.1
	Viscosity is markedly increased (not due to number of red cells)	Viscosity is not increased, although the number of red cells is large

THE DETERMINATION OF THE ALBUMIN AND GLOBULIN PERCENTAGE IN THE SERUM

In discussing the viscosity of the serum as a factor of the total value, it has been mentioned that viscosimetry furnishes approximately the percentage of protein content of the serum. Large series of experiments have shown that there are discrepancies which can only be explained by the differences in viscosity of various proteins, because for the same amount of protein different viscosimetric values have been obtained. The explanation has been furnished by the experiments of Heyder and Rohrer, who showed that the protein consists of two substances of different viscosity, albumin and globulin; the former less viscous than the latter. It is therefore to be expected that a solution of protein rich in albumin should have a lower viscosity than a solution of the same percentage of protein rich in globulin. Heyder and Rohrer have established a definite relation among the three factors: viscosity, protein percentage, and albumin-globulin mixture. They established the viscosimetric curve of albumin solutions of different concentration and one of globulin solutions under the same conditions. The viscosity was measured with the viscosimeter of Hess, and the concentration with Pulfrich's refractometer, which, according to Naegeli, permits a very accurate determination, requiring only a few drops of serum (for notes on handling the apparatus see instructions with it).

The albumin and globulin curves determine a field which includes all possible rates of any mixture of albumin and globulin. The important part of this field is reproduced in Fig. 3, by means of which it is possible to interpolate the unknown factor, the albumin-globulin mixture in percentage. Charlotte Loebner, in her study of the serum conditions in carcinoma, describes a method of obtaining a small quantity of serum. Twenty drops of blood are collected in a test tube 5 cm. long and 1.5 cm. in diameter; the tube is corked and put in an ice box for a few hours. The serum is aspirated with a thin pipette, the point of which is deflected in a right angle. If the serum is not perfectly clear, it must be centrifuged. Naegeli provides the following data:

"The serum protein in normal individuals contains 60 to 80 per cent albumin and 20 to 40 per cent globulin.

"The relation A:G (albumin to globulin) is almost constant during the day.

"No difference exists between arterial and venous blood.

"Intake of food and water does not change the relation A:G.

"Muscular activity has no influence on the albumin-globulin ratio.

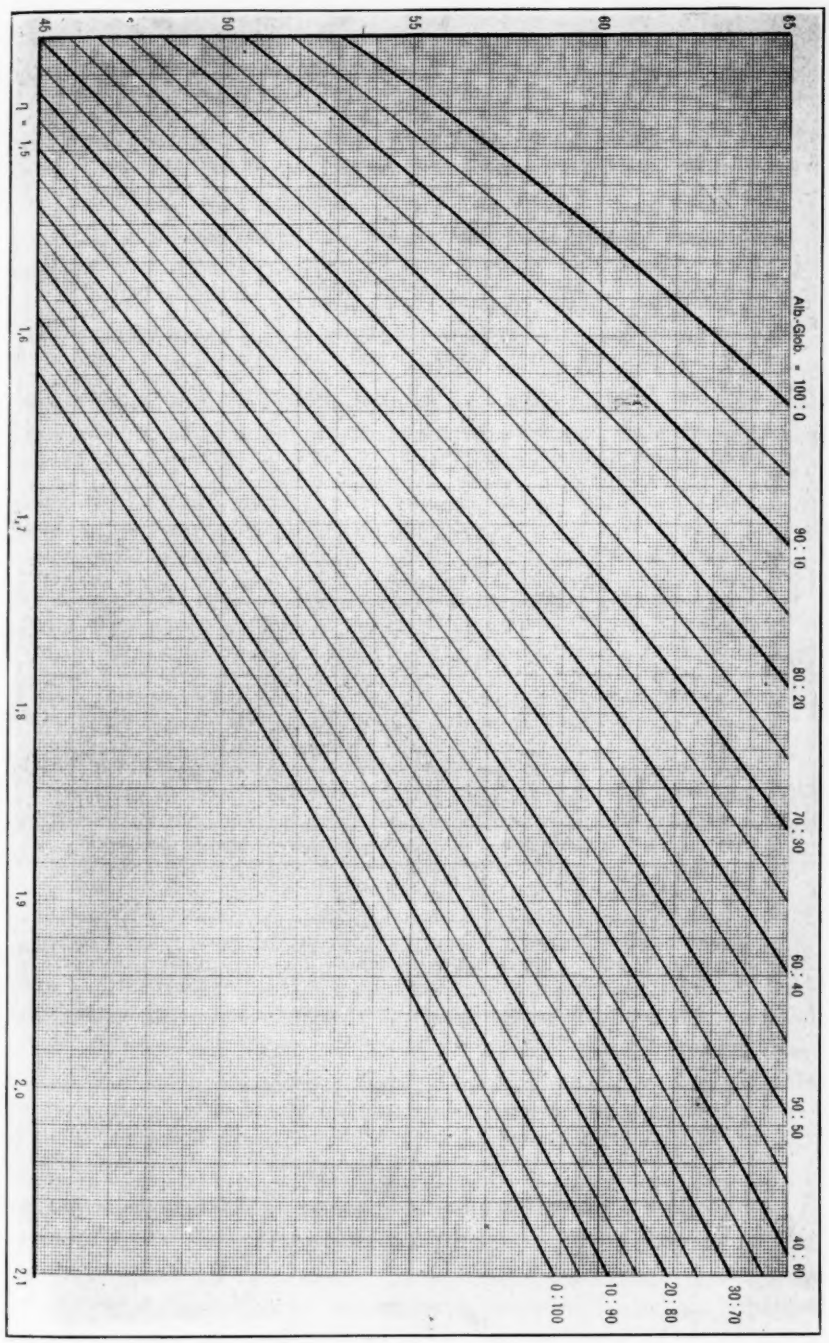

Fig. 3.—Viscosimetric and refractometric function of albumin-globulin mixtures (Chart used at the clinic of the University of Zurich). This chart allows the interpretation of an unknown mixture of albumin and globulin when the viscosity and the refraction of the serum have been determined.

"The newborn have a low protein content and low globulin percentage. There is a marked difference between the blood of the mother and the blood of the child.

"The proportion of albumin to globulin shows the following deviations in pathologic conditions:

"Hunger, diseases of the heart and lungs, and cachexia: The globulin content in venous blood is increased in comparison with the globulin content in the arterial blood.

"Chlorosis: In extreme hydremia no change in the A:G relation can be found. Although the protein content of the serum increases after treatment, the relation A:G remains unchanged. Any deviation from this rule puts the diagnosis of chlorosis in doubt or complications must be considered.

"Pernicious anemia: In progressive cases the albumin content steadily increases.

"Secondary anemia (carcinoma or tuberculosis): The progress of the disease is accompanied by a marked increase of the globulin factor."

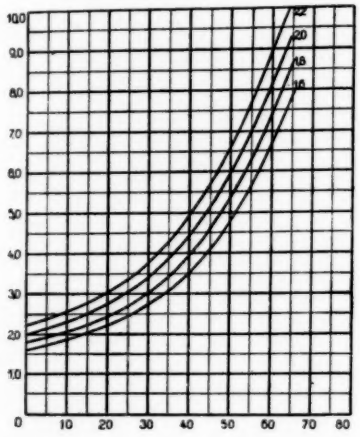

Fig. 4.—(From textbook of O. Naegeli). Viscosimetric function of the red blood cell concentration of fresh blood. The volume of red blood cells for each unit of blood can be determined by means of this chart when the viscosity of whole blood and of the suspending plasma is known.

The albumin globulin proportion of the blood serum in syphilitic patients has been studied by this method. The results will be reported soon by Bircher and MacFarland.

THE DETERMINATION OF THE TOTAL BLOOD CELL VOLUME AND THE EXACT VOLUME OF A SINGLE RED CELL

A hyperbolic curve was obtained in measuring the viscosity of blood samples which were so arranged that the first consisted of serum only and the following samples had a steadily increasing amount of suspended red cells. With serums of higher protein concentration similar curves were almost absolutely parallel with the first one. Figure 4 shows a series of parallel curves starting at different viscosimetric values according to the viscosity of the suspending serum.

To determine the total cell volume it is only necessary to know the vis-

cosity of the blood and the viscosity of the plasma. The viscosity of the plasma η_2, indicates which curve has to be chosen (point η_2) and the viscosity of the whole blood η will be found lying on this curve. The corresponding point on the abscissa indicates the percentage of the cells. By an easy interpolation it is possible to cover every case. It is advisable to use plasma instead of serum, because the plasma constitutes the real suspending fluid for the red cells. Naegeli advises adding to the blood some Hirudin crystals immediately after withdrawal, and separating the plasma from the cells by sedimentation or centrifugalization. This method is not absolutely exact, as I have been able to show, but compared with any other clinical method the difference is not more than $\pm$ 3 per cent (Alder-Naegeli).

To get the volume of one red cell it is necessary to divide the total volume of red cells in 1 c.mm. of blood, as determined by this method, by the number of cells. A total volume of red cells of 0.44 c.mm. is found in 1 c.mm. blood containing 5,000,000 cells and therefore one red cell equals 0.44: 5,000,000, equals 88 cubic micra, or, to simplify the calculation, the volume figure of 1 c.mm. blood is multiplied with 1000 and divided by the cell number expressed in millions, for example

440:5 equals 88.

Considering the platelets and the white cells, this figure has to be reduced to 87 cubic micra or 86 cubic micra.

TABLE III

Data on pathologic subjects (Naegeli)	
Cured chlorosis	78 to 83 (still too low)
Secondary anemias	59 (lowest rate)
Hemolytic anemias	92
Pernicious anemias	165 to 120

In relation to cardiovascular diseases the hydrodynamic significance of the constitution of the blood should be considered with much more attention than it has received. Einstein and Hess have repeatedly discussed the problem and have given definite formulas for the hydrodynamic influence of the total cell volume. In an elaborate study Hess[31] showed the relation between the heart work and the red cells. He states that not the amount of blood thrown out by the heart but the load of cells and hemoglobin carried by the blood is the index of the effect of heart work. A greater volume of red cells for each unit of blood volume is more effective than a smaller volume, but the viscosity, being opposed to the heart work, increases accordingly and these two opposite effects reach a point of equilibrium, where viscosity counterbalances the effective load. This point has been calculated theoretically by Hess and called the optimum phase. (It lies between 40 per cent and 50 per cent red cell volume.) This same optimal condition exists in normal blood.

The knowledge of the volume percentage is as valuable for the functional examination as for diagnostic purposes. This point has been discussed by Naegeli, whose findings are given in Table IV.

TABLE V

	HEMOGLOBIN PER CENT	RED CELLS MILLIONS	FORMED ELEMENTS, VOLUME PER CENT	VISCOSITY
		Chlorosis		
Chlorosis	60	4.56	33.5	3.4
Chlorosis	78	4.272	30.9	3.1
Cured chlorosis	99	4.60	40.9	4.2
Cured chlorosis	97	4.58	40.7	4.0

The number of red cells is normal, but the percentage volume is decreased. The red cells are more or less collapsed because of their insufficient filling with hemoglobin. Nothing is more interesting than to watch by this method the gradual filling out of the cells. The cured patients show normal blood cells with their relative viscosity at a normal rate.

		Anemia		
Secondary anemia	37	2.03	17.9	2.3
Hemolytic anemia	52	2.56	23.6	2.7
Pernicious anemia	44	1.08	17.9	2.4
Pernicious anemia	96	3.57	43.1	4.3

In secondary anemia red cells, percentage of volume, and viscosity decrease at the same rate; there is no change in the size of the red cells.

In pernicious anemia the percentage of volume may be found to be the same as in secondary anemia, while the number is only one half. This is clearly indicated by the viscosity.

SOME VISCOSIMETRIC DATA IN RECENT LITERATURE DEMANDING FURTHER INVESTIGATION

The viscosimetric factor of the blood has been studied in the following conditions:

1. *Morbus Basedowii (exophthalmic goiter).*—Kaess found the following variations: (a) Decreased viscosity in pure sympathicotonic cases. (b) Increased viscosity in pure vagotonic cases. (c) Inconstant variations in mixed conditions. (d) Return to normal rates about three weeks after successful operation.

2. *Pregnancy.*—Pellisier found that in (a) pregnancy with albuminuric complication a decrease in viscosity and a simultaneous increase in blood pressure indicate an obstruction of the kidneys; and that in (b) pregnancy with tuberculosis, with low arterial tension, an increase in viscosity indicates a serious form of disease.

3. *Infancy.*—Weill and Gardére believe in the prognostic value of the viscosity in severe nutritional disturbances. They found (a) decreased viscosity in anemia and eczema and (b) increased viscosity in pneumonia and congenital cardiac lesions.

4. *Surgical Diseases.*—Very similar results have been obtained by Bolognesi, Müller, Oehlecker, Simon, Süssenguth, Frischberg, and Mayesima.

A. The normal course of the viscosimetric factor in aseptic operations (Müller): (a) Postoperative increase (reaches the highest value the first day). (b) Gradual decrease in the following days. (c) Fall below the normal (phase of exhaustion). (d) Regaining the normal rate (phase of recovery).

B. Fracture of the skull (Oehlecker). The injury of the marrow of the

bones is followed by an increase of the viscosity of the blood. This is therefore an aid in the differential diagnosis of the injuries of the head.

C. Appendicitis (Simon). An increase in viscosity demands an immediate operation. In acute cases an operation should be performed regardless of a normal viscosity (Table V).

D. Differential diagnosis of rupture of extrauterine gravidity, perityphlitis, involvement of the adnexa uteri (Oehlecker, Simon, Süssenguth). (a) Low values point to involvement of the tubes or ovaries and may exclude appendicitis. (b) Extremely low values indicate internal hemorrhages, for example, after rupture. (c) High values are found in perityphlitis.

E. The question of drainage of the abdominal cavity (Oehlecker). The increased viscosity points to drainage of the abdominal cavity after operation, even if the pathologic changes do not seem to be dangerous.

F. Prognosis (Oehlecker, Simon, Süssenguth). (a) A steady decrease of the viscosity in secondary anemias denotes a poor prognosis. (b) If in peritoneal infections viscosity does not decrease after operation, the danger is not removed, or complications are developing. (c) In most of the cases of peritonitis an increase in viscosity indicates death.

5. Normal viscosimetric values as a standard for comparison with the rates obtained in acute surgical cases (Hess[23]).

TABLE V

AGE	VISCOSIMETRIC RATES	
	MALES	FEMALES
0 to 10 years	3.8	3.8
10 to 20 years	4.4	4.2
20 to 35 years	4.7	4.2
35 to 50 years	4.9	4.4
50 to 81 years	4.6	4.5

SUMMARY

The problem of viscosity of the blood was taken up by Poiseuille in 1847 and, though a considerable amount of work has been done since then, the medical profession, especially in America, has paid little attention to it.

The value of determining the viscosity is threefold:

1. In circulatory questions, viscosity should be considered. Red cells and viscosity are the two opposite factors which have a determining influence on the effect of heart work.

2. The viscosimetric factor is an excellent check of the normal blood test, because it depends on the different constituents of the blood. It is a delicate indicator for changes in their relation, and on this fact are based some valuable diagnostic information.

3. The viscosity is an accurate means of analyzing the constituents of the blood: the quantity and quality of protein dissolved in plasma may be studied as well as the exact volume of the cellular elements. On these determinations are based the diagnostic applications supported by Naegeli.

A tabulation of some valuable findings is given with the intention of promoting further studies along this line.

The viscosimeter of Hess has overcome all the difficulties necessarily adherent to the determination of a dynamic factor in a living body. The test requires one drop of blood, and is easily carried out in thirty seconds, giving an accuracy of clinical value.

The viscosity of blood is compared with the viscosity of distilled water under the same conditions and is therefore relative. The normal values depend on age, sex, and constitution: the average is 4.5.

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THE TREATMENT OF ACUTE PHOSPHORUS POISONING*

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PHOSPHORUS poisoning is at present not so frequent, consequently not so important as formerly, yet it does occur, and the present investigation was instigated by a recent case of phosphorus poisoning successfully treated, by a method which involved the theories discussed in this paper, on the service of Dr. Charles Spencer Williamson in Cook County Hospital. Since red phosphorus is practically nontoxic, the statements which follow apply only to yellow phosphorus. Yellow phosphorus is only slightly soluble in water, absorption takes place relatively slowly, and elimination is also retarded, phosphorus having been found by Starck in the feces three and one-half days and in the vomit two days after the ingestion of a fatal dose. For these reasons the stomach tube, emetics and purges may be useful during the first day and many writers recommend them. The use of emetics seems superfluous. Blood transfusion and the oxidation of the phosphorus with potassium permanganate, hydrogen peroxide, oil of turpentine and copper sulphate have been tried (Sollmann, Hare, Kobert). The effectiveness of all these treatments has been repeatedly questioned. Old "ozonized" French oil of turpentine may be useful but rectified German and American turpentines are ineffective. Dilute copper sulphate envelopes the globules of phosphorus with a coating of oxidized copper which retards absorption. Copper sulphate also acts as an emetic if given in excess but studies by Thornton and Hare indicate that when given in doses large enough to be effective, it is as dangerous a poison as the phosphorus. This is probably due to a depression of the emetic mechanism, for a similar lack of response to catharties is often noted in cases of paresis of the bowel in infections, etc., consequently lavage is indicated in all such cases since emetics and catharties may be ineffective, and cannot be so efficient as lavage. When an oxidizing agent is used in phosphorus poisoning, it is usually recommended that it be followed by a saline purge such as magnesium sulphate or magnesium citrate. Sodium bicarbonate is recommended to prevent acidosis. Magnesium citrate may be useful to combat acidosis since it is the salt of an organic acid and will be oxidized to a carbonate if absorbed. It is recognized that when the systemic symptoms set in, these methods are practically useless and the treatment must be mainly symptomatic.

Warning is given by most all authors to avoid oils, fats and milk while any phosphorus remains in the alimentary tract, since they increase its solubility and rate of absorption either along with the fatty derivatives formed by the lipases of the duodenum or, due to the precipitation of the phosphorus

*From the Laboratory of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago.

on the walls of the gut during the absorption of the fatty menstrum in such a finely divided condition that the large surface exposed facilitates absorption thus hastening the fatal termination of the case.

I thought that advantage might be taken of the relatively high solubility of phosphorus in oils in the treatment of acute phosphorus poisoning, especially since it extends also to the mineral oils. It was thought that castor oil might dissolve phosphorus and since castor oil is but slightly absorbed it could be used to sweep the phosphorus out of the alimentary canal before any appreciable absorption had taken place. Only a few experiments were undertaken with castor oil however because of the well-known fact that dogs are hard to purge with castor oil and because it is absorbed by dogs to a considerable extent. It is also used by human beings notably the Chinese who use it as a food fat. This, however, is not an absolute contraindication, since, if used with other cathartics, absorption would be lessened. Cottonseed oil also dissolves phosphorus quite readily and attempts were made to take advantage of this fact first and then sweep the resulting solution out of the body with a saline cathartic. These attempts were not very successful because of the apparent paresis of the bowel. The theory, however, is valid and unquestionable if any cathartic will act, and there is no reason to assume that if castor oil, which is an excellent solvent for phosphorus, be used in addition to a saline cathartic, the results would be more efficient than if saline alone were used. However we have a more reliable treatment, consequently there is little gained in pushing this theory to the limit. This treatment was found to be the use of the mineral oil, liquid petrolatum, which is not absorbed, consequently it has only a benign influence.

METHODS

Dogs were selected of about the same size (10 to 12 kilograms) and fed for several days before being given phosphorus which was administered in solution with oil or carbon disulphide on an empty stomach. Twenty-five dogs in all were used. The following protocols give the procedure followed:

SERIES A.—Two dogs were given 0.5 gram of phosphorus dissolved in cottonseed oil; two more were given the same dose of phosphorus dissolved in 50 c.c. castor oil and two more the same dose dissolved in 50 c.c. of liquid petrolatum. Defecation took place in all but the first two. The first four died in from one to three days and the two given liquid petrolatum are still living after one month without impairment of appetite or harmful symptoms except for the first three hours.

SERIES B.—Five dogs (1) 1 c.c. of carbon disulphide alone (2) 0.5 gram phosphorus dissolved in 1 c.c. carbon disulphide (3) the same dose as in No. 2 but followed one hour later with one ounce of magnesium sulphate, (4) the same dose as in No. 2 but followed in one hour with 50 c.c. of castor oil, (5) the same dose as No. 2 but followed in one hour with 100 c.c. of liquid petrolatum. All dogs defecated either spontaneously or under the influence of the cathartic. The control dog No. 1 and the dog given liquid petrolatum alone survived as did two additional dogs treated as No. 5 on the following day.

SERIES C.—Four dogs were each given 0.5 gram phosphorus dissolved in 10 c.c. of cottonseed oil and after 40 minutes two were given one ounce of magnesium sulphate and two 50 c.c. of liquid petrolatum. Defecation occurred in all four dogs. The first pair survived for two days and the second pair for four and one-half days.

In Series A and B it is evident that neither castor oil nor magnesium sulphate prolong the life of a dog receiving a fatal dose of yellow phosphorus, but that liquid petrolatum given one hour after taking phosphorus furnishes complete protection against the onset of harmful symptoms. In Series C it is evident that liquid petrolatum prolonged the life of the dogs for two days even when the phosphorus had been given dissolved in a small volume of cottonseed oil, but that the cottonseed oil had facilitated the absorption of the phosphorus to such an extent that death was delayed only.

Liquid petrolatum is physiologically inert and acts entirely by reason of its physical properties. Its use is recommended in the treatment of phosphorus poisoning. Since liquid petrolatum is a harmless, and nonirritating cathartic, it may be used to delay absorption from the gut in many, perhaps all cases, of poisoning.

In the experimental work, I have not investigated the value of lavage. This seems to me to rest on such solid ground that in addition to any other treatment it should be most relied on, and most thoroughly used. Petrolatum could be used advantageously also in the lavage.

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THE SYNTHESIS OF ARSPHENAMINE AND A STUDY OF SOME OF ITS INTERMEDIATE DERIVATIVES*

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INTRODUCTION

ARSPHENAMINE is the name under which the synthetic arsenic compound, formerly known as "salvarsan" or "606," is now licensed to be manufactured in the United States.

Previous to the outbreak of the war in Europe, the entire world's supply of this drug was controlled by German manufacturers. At present, it is being made in France, England, Japan, Russia, Canada and the United States. Its production on a commercial scale was begun in this country in the latter part of 1916, and a sufficient quantity is now being turned out to meet our immediate needs. The American chemist has not only mastered the details of the process of manufacturing this material on a commercial scale, but also he has improved the quality of the product to such a degree that the present output is in most cases therapeutically equal to the original German stock. The conditions responsible for these achievements are briefly described in the paragraphs immediately following because of their importance as factors which led to the preparation of this paper.

Almost simultaneously with the beginning of hostilities in 1914, German shipments of salvarsan to this country began to fall off, and long before our entry into the conflict, they had ceased entirely. Fortunately for us, the manufacture of the drug was begun in England, France, Japan and Canada before this last stage was reached, and we were able to secure some of the material from these sources. The quantity available, however, was limited to that produced in excess of the home needs of these countries and their allies and small quantities produced in this country; this was far too small to supply our needs. This shortage became acute when we entered the conflict and the mobilization of our forces was begun. It then became necessary to supply the demands of our Army and Navy at the expense of the civilian population or to undertake the manufacture of the drug on a large scale.

The problem of devising a process of manufacture suited to conditions in this country was not unattended with difficulties as our chemists had little or no previous experience in the preparation of arsenic compounds. To be sure, there was a great amount of information on the subject in the chemical literature and the patent specifications were available. In fact, the entire

*This paper was prepared in 1918 and was intended to furnish impartial information to the large numbers of consumers who were seeking details to assist them in the clinical use of the arsphenamine. Paper shortage and delays postponed the early publication of the article.

The author wishes to give due credit to Dr. G. C. Lake for biological data and to Dr. A. G. DuMez for chemical data used in this article.

process of manufacture is described in the various numbers of the "Berichte der deutschen chemischen Gesellschaft." Production was further delayed by difficulties experienced in securing the necessary equipment for manufacturing on a commercial scale, and by the necessity for changes in the process brought about by the prohibitive cost of certain essential chemicals. Nevertheless, such rapid progress was made that on November 30, 1917, the Federal Trade Commission issued orders for licences to manufacture the drug to no less than three manufacturers, namely, the H. A. Metz Laboratories, Inc., New York, the Dermatological Research Laboratories, Philadelphia, and the Takamine Laboratory, Inc., New York.

As already mentioned in the beginning of this paper, the drug received in this country before the war was produced solely in Germany, where it was manufactured under the direct supervision of its discoverer, Ehrlich. It was carefully tested, both chemically and physiologically, to establish its identity and to determine its degree of toxicity. Naturally, a fairly uniform product was obtained. With the advent on the market in this country of the various brands produced by the newly established factories in the countries previously mentioned and the assurance that American manufacturers would soon be producing, it became necessary to establish standards for the drug in order to insure a product of good quality and to guard against the appearance of spurious material. This the United States Public Health Service undertook to do, and on November 22, 1917, tentative rules and regulations for the manufacture and sale of the drug were promulgated. (See Public Health Reports, 1917, v. 32, No. 49, p. 2071).

In order that the Public Health Service might keep in as close touch as possible with developments in the process of manufacture and be of the greatest assistance in aiding the manufacturers to improve the quality of their respective products, it was highly desirable that a representative be stationed at one of the manufacturing plants. The fulfillment of this desire was made possible through the courtesy of H. A. Metz of the H. A. Metz Laboratories, Inc., New York, who offered the Public Health Service the use of his plant gratuitously. As a result, Dr. C. N. Myers was detailed to this plant to make a study of production on a commercial scale and to supply the Hygienic Laboratory of the United States Public Health Service with the materials, including intermediates, by-products, et cetera, necessary for carrying out such pharmacological and toxicological studies as might have a bearing in this connection.

This paper deals with some of the results of these studies insofar as they pertain to the process of manufacture and the toxicity of a few of the compounds isolated or prepared by the author.

MANUFACTURE OF ARSPHENAMINE

The processes for the manufacture of arsphenamine herein described are intended to apply to the preparation of a product identical with that originally made in Germany under the supervision of Ehrlich. This statement is made because of the fact that the physical and chemical properties of some of the

products now on the market indicate that they differ from the original in their make-up.

The mother substance, or material which yields arsphenamine, when reduced under the proper conditions, is 3-nitro-4-hydroxyphenylarsinic acid, commonly known in the industry as "nitro oxy." There are three well-defined methods of preparing this compound in use. Descriptions of these methods follow.

Method 1.—The following is perhaps the most convenient method for preparing "nitro oxy" because of the ready accessibility of the materials in normal times.

Mix 25 g. of arsenous chloride with 15 g. of dimethylaniline and, after the first heat of reaction is over, heat at the temperature of a water-bath for two hours. Pour the syrupy liquid into ice-cold water and treat with an excess of an aqueous solution of caustic soda until the p-dimethylaminophenylarsenous oxide is dissolved. The small amount of hexamethyltriaminotriphenyl arsine formed is filtered off and the unaltered dimethylaniline removed with any suitable immiscible solvent, such as petroleum ether. The filtrate is then treated with 30 per cent hydrogen peroxide, and the p-dimethylaminophenylarsinic acid precipitated by means of acetic acid (Michaelis, 1908).

This arsinic acid is sparingly soluble in cold water or alcohol, dissolves readily in hot alcohol, hot dilute acetic acid, mineral acids or alkalies. It crystallizes in needles from hot water and hot alcohol, and sublimes without melting. It forms a sodium salt (N-dimethyl-atoxyl) which corresponds to the formula $(CH_3)_2NC_6H_4AsO(OH)ONa.5H_2O$.

Suspend 23.5 g. of the finely powdered p-dimethylaminophenylarsinic acid in 300 c.c. of glacial acetic acid and effect solution by the addition, at ordinary temperature, of 9.8 g. of 62 per cent nitric acid. Upon the addition of 20 c.c. of acetic anhydride, the solution becomes yellow and deposits a heavy precipitate consisting of pure 3-nitro-4-dimethylaminophenylarsinic acid. By warming the latter with 2.5 parts of 40 per cent aqueous solution of caustic soda at 85° C. for 3-4 hours, the corresponding hydroxy compound, "3-nitro-4-hydroxyphenylarsinic acid or nitro oxy," is obtained.

Contamination of the "nitro oxy" with the nitro-dimethylamino compound mentioned above should be avoided as the latter yields 4:4′-tetramethyl-3:4-3′-4′-tetraminoarsenobenzene in the final stages of reduction with alkaline hydrosulphite in the presence of magnesium chloride. Upon subsequent treatment with methyl muriate, the tetrahydrochloride is formed (Karrer, 1913).

An alternative and preferable method of nitrating the dimethylamino-arsinic acid consists in dissolving 100 g. of the material in 250 g. of 60 per cent sulphuric acid and slowly adding a mixture of nitric acid (sp. gr. 1.49), 35 g. and sulphuric acid (60 per cent), 150 g., allowing the temperature to rise to 30° C. This temperature is maintained for half an hour or longer and the mixture poured on broken ice. Dissolve the yellow precipitate in aqueous sodium carbonate, filter, and precipitate the nitro-compound by the addition of a very dilute mineral acid. After purification by crystallization from hot water, the compound is hydrolyzed as described above yielding "nitro oxy."

The foregoing nitration may be carried on in concentrated sulphuric acid by keeping the temperature below 10° C.

If the nitration is carried out in more dilute solutions, or if sodium nitrate in sulphuric acid (1 to 4) is added to p-dimethylaminophenylarsinic acid dissolved in sulphuric acid (1 to 4) and heated to 90° C., one obtains 4-methylnitrosoaminophenylarsinic acid. On the other hand, a dinitro-compound will be obtained if there is an excess of nitric acid used and the dilution of the solvent is about 1 to 4. This process yields chromo isomerides in which there is an

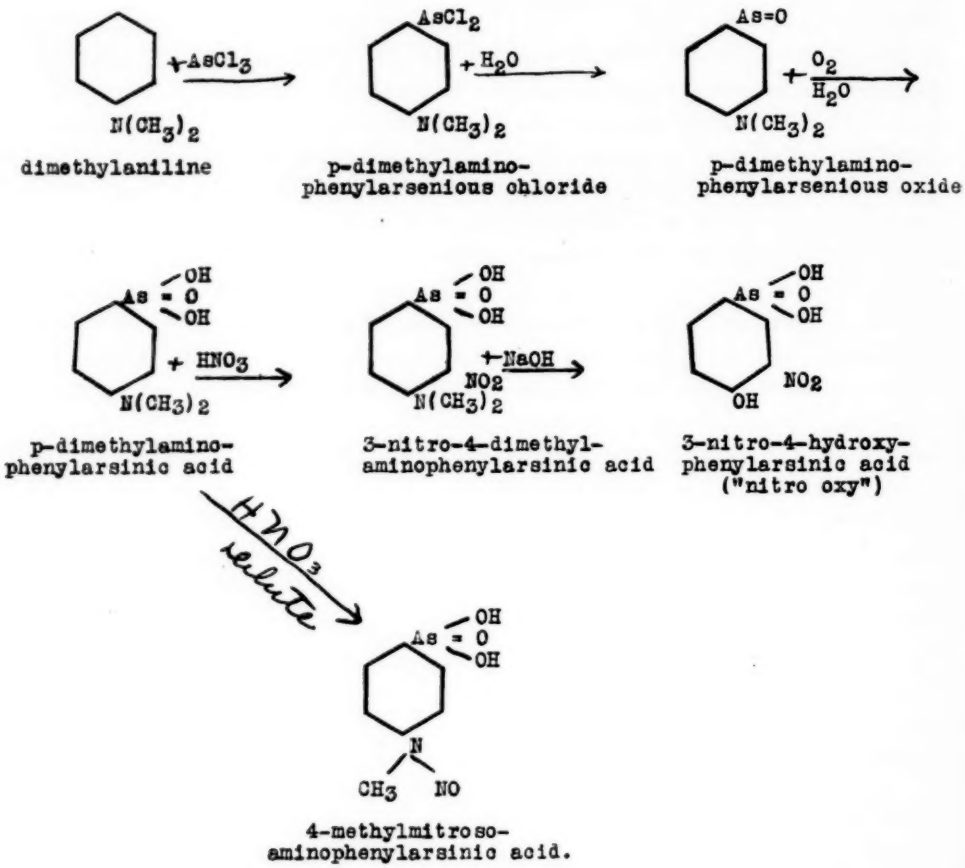


Fig. 1.

orientation of the substituent groups or in which the nitro groups occupy different positions in the aromatic nucleus. One isomer dissolves in aqueous sodium carbonate, hot water, alcohol, or acetone, separates in rosettes of bright yellow, prismatic crystals, and melts with decomposition at 161° C. The other yields small, red, four-sided plates, less soluble in hot water than the yellow modification and melts with decomposition at 158° C.

Advocates of the above method of preparing "nitro oxy" claim a very smooth nitration of the p-dimethylaminophenylarsinic acid leading to a series of definite compounds which are not always obtained by the circuitous routes

employed in the other methods. Further, this method has additional advantages in that (1) p-dimethylaminophenylarsinic acid is readily prepared from easily accessible reagents, dimethyl aniline and arsenous chloride; (2) the dimethylamino complex is removable quantitatively by hydrolysis, the products being O-nitrophenolarsinic acid (3-nitro-4-hydroxyphenylarsinic acid or "nitro oxy").

The structural formulas in Fig. 1 represent the general changes involved in the formation of "nitro oxy" by this method.

Method 2.—Another procedure which may be followed in making "nitro oxy" consists in stirring together 94 parts of phenol and 151 parts of crystallized arsenic acid and heating this mixture at 150° C. for four hours. The mass, which becomes dark colored, is extracted with warm water when cool and the filtered solution is concentrated *in vacuo*. The concentrate is extracted repeatedly with acetone, and the solvent is finally evaporated leaving crude phenol-p-arsinic acid as an oily liquid which gradually solidifies. The acid is purified by crystallization from glacial acetic acid, water, alcohol, acetone or dilute mineral acids. It readily forms salts with alkalies, and purification may be effected by the formation of an alkaline salt and subsequent treatment with a concentrated mineral acid (Farbwerke vorm Meister Lucius and Brüning, 1909).

This acid may also be prepared by the Bart process in which p-aminophenol is diazotized in hydrochloric acid and warmed with alkaline sodium arsenite. The solution is neutralized, filtered, boiled with animal charcoal, and concentrated, yielding sodium phenol-p-arsinate. The acid is precipitated from the salt by a mineral acid, preferably hydrochloric acid (Bart, 1912).

A third method by which the acid may be obtained consists in diazotizing a hydrochloric acid solution of p-arsanilic acid, evaporating to dryness and extracting the free acid formed with acetone (Barrowcliff, Pyman and Remfry, 1908).

To prepare "nitro oxy" from the above acid, a mixture of 39 c.c. of nitric acid (sp. gr. 1.4) and 39 c.c. of concentrated sulphuric acid is added slowly to a solution of 144 g. of the sodium salt dried at 80° C. and dissolved in 450 c.c. of concentrated sulphuric acid at 0° C. The temperature is kept between 0° and 5° C. The temperature after the addition of the nitric acid is not allowed to rise above 10° C. The acid solution is finally poured into 2500 c.c. of cold water and the precipitated "nitro oxy" collected after 24 to 48 hours (Benda, 1911). Careful work is necessary in this procedure as too energetic nitration leads to the formation of the dinitro-compound (3:5-dinitro-4-hydroxyphenylarsinic acid) which may be detected by the red color obtained with alkali and sodium hydrosulphite. High temperatures are to be avoided for the same reason. The yield of "nitro oxy" by this procedure never amounts to more than 75 per cent of the theory.

Because of the fact that the dinitro-compound was prepared for experimental purposes, a description of the method by which it was actually made is given at this point. Twenty grams of dry phenol-p-arsinic acid were added in small portions to 60 c.c. of concentrated sulphuric acid cooled to 0° C. by using a

freezing mixture. To this mixture 13.4 c.c. of nitric acid (sp. gr. 1.52) were added drop by drop with constant stirring, keeping the temperature between 15° C. and 20° C. The stirring was continued for two hours at ordinary temperature. The mixture was then poured on 300 grams of ice and by scratching the container and cooling to 10° C., the compound separated out as lustrous, pale yellow leaflets (Benda, 1912).

The dinitro-product may also be obtained from 3:5-dinitro-p-arsanilic acid. The acid is heated with 10 per cent caustic potash at 90° C. until the ammonia is eliminated, when the dinitro-compound is precipitated by the addition of hydrochloric acid.

The following structural formulas (Fig. 2) explain the important chemical changes which occur in the preparation of "nitro oxy" from phenol.

Method 3.—A third method for the preparation of "nitro oxy" is based

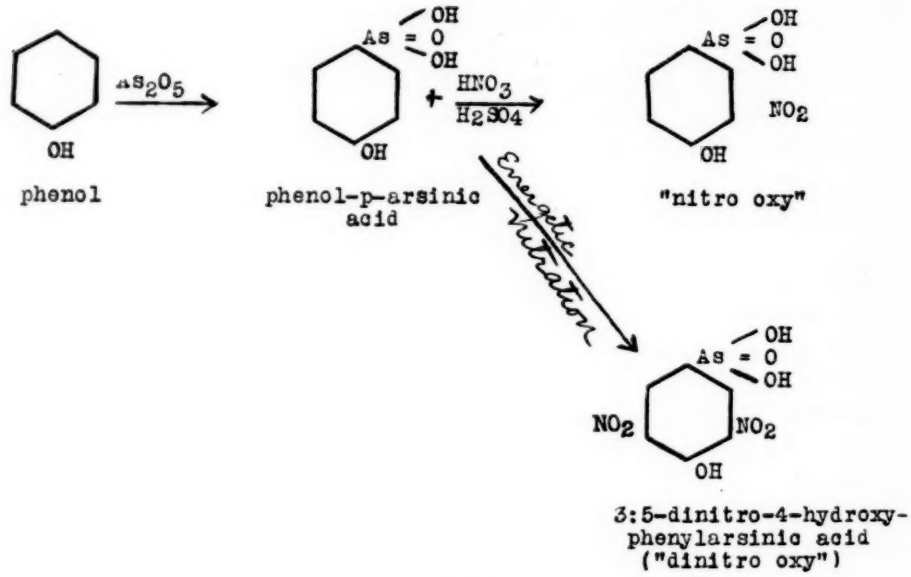


Fig. 2.

on the use of aniline as the material for beginning the synthesis. The aniline may be arseniated by either of the following procedures.

The direct combination of arsenic and aniline is effected by heating in a suitable container crude arsenic acid (containing 75 per cent or more As_2O_5) until it becomes practically 100 per cent As_2O_5. Aniline (186 grams) is cooled to about 0° C. and 140 grams of the dry arsenic acid is slowly added with constant stirring. The mixture soon thickens and finally a crystalline mass forms. At this stage the stirring should be vigorous enough to pulverize the material. The composition of this product is probably an arsenate of aniline. The temperature is slowly raised to 170° C. with continuous stirring, allowing the powder to melt slowly. The apparatus in which this operation is carried out should be equipped with an oil bath and a condenser to dispose of the aniline which is volatilized. Finally the temperature is raised to 190° and main-

tained with continuous stirring for two hours. The product is then mixed with water, rendered alkaline, and the excess of the base distilled off in steam. The residue is allowed to cool, filtered, concentrated, and neutralized with hydrochloric acid, when the crude arsanilic acid separates out. This product is dissolved in aqueous caustic soda to a faintly alkaline reaction. At this stage, the material is sometimes boiled with animal charcoal (usually the free acid is boiled with charcoal as there is less danger of splitting off the arsenic) and filtered into ethyl alcohol, when the Sodium Salt (atoxyl) separates out. The impurity, which is secondary arsanilic acid or di-4-amino-diphenylarsinic acid, remains in solution. The free arsanilic acid is liberated from atoxyl by means of dilute hydrochloric acid (Adler, 1908). This procedure for combining arsenic and aniline is known as Béchamp's condensation (Béchamp, 1863). It is sometimes spoken of as the melt method.

Para arsanilic acid is sparingly soluble in ethyl alcohol, water, more so in methyl alcohol, insoluble in ether and the other organic solvents. It is amphoteric, dissolving in excess of mineral acid, and being reprecipitated by sodium acetate. It is partially hydrolyzed by boiling with water. The product in its purest condition is a white crystalline solid. Occasionally it has a slight pink to violet color.

Secondary arsanilic acid is a colorless, needle-like solid having a melting point of 248-249° (232°). It is sparingly soluble in water or the ordinary organic solvents, moderately soluble in glacial acetic acid, readily soluble in ethyl alcohol, dilute mineral acids in excess, and in dilute alkalies. Methods for the preparations of this compound are described by Pyman and Reynolds (1908) and Benda (1908). (Fig. 3.)

A second method of making arsanilic acid from aniline consists in preparing acetanilide and subsequently the para nitro-compound. The latter is reduced to acetphenylenediamine, arseniated by means of arsenous acid, and diazotized with sodium nitrite yielding the corresponding acetyl derivative of arsanilic acid. The acetyl group is finally removed by hydrolysis leaving the crude arsanilic acid. The product thus obtained is impure, being accompanied by tarry residues and diazo derivatives. The removal of these impurities is absolutely necessary to insure success in subsequent operations. This is accomplished as described above.

The para arsanilic acid obtained by either of the above methods may be converted into "nitro oxy" by direct nitration of hydroxyphenylarsinic acid, which is first prepared, or by nitration of the oxalyl compound. The latter procedure is preferable as it yields only mononitro-compounds, whereas some of the dinitro-compound is usually formed when direct nitration is resorted to.

In the first instance, the arsanilic acid is dissolved in concentrated sulphuric acid and diazotized with the molecular equivalent (1 molecule) of sodium nitrite, the diazo solution filtered and the filtrate heated to about 70° C. The hot acid solution is then treated with barium carbonate, filtered and a small amount of sodium sulphate and animal charcoal added. The solution is then concentrated to effect crystallization. The compound obtained is the mono-sodium salt of phenol-p-arsinic acid (Bertheim, 1908, p. 1853). The free

Aniline Method No. 1.

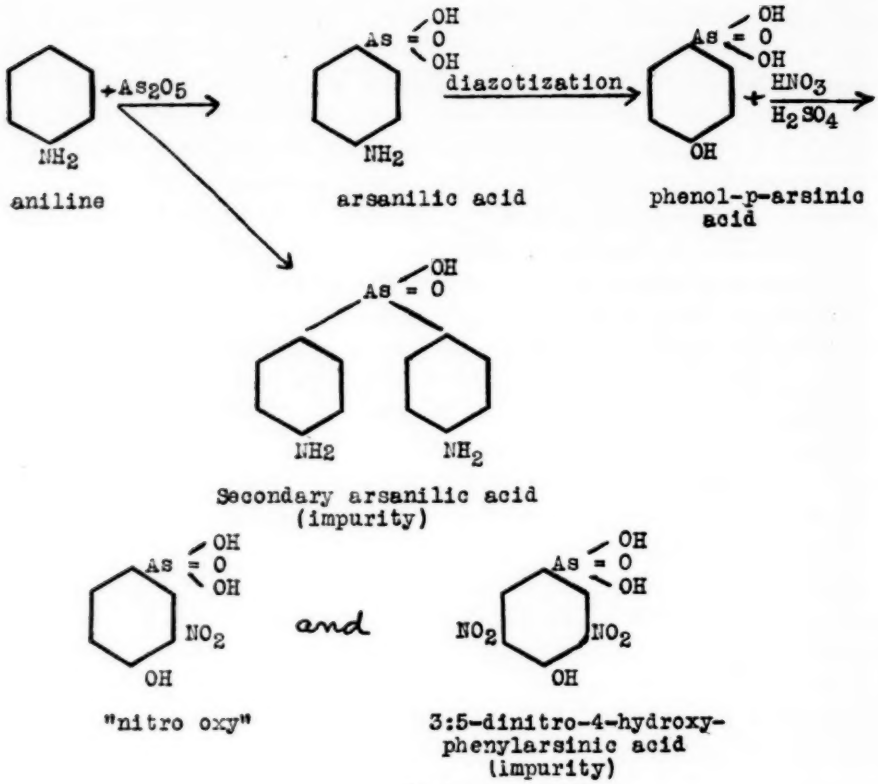


Fig. 3.

acid is liberated by means of a mineral acid. The latter may be obtained directly by carrying out diazotization in hydrochloric acid as described under method 2. Direct nitration of the diazotized compound by means of a mixture of nitric and sulphuric acids yields "nitro oxy."

When nitration is effected through the oxalyl compound the procedure is as follows: Thoroughly mix 347 g. of crystallized sodium p-arsanilate with 378 g. of crystallized oxalic acid, and heat at a temperature of 120° to 130° C. until the greater part of the water is eliminated. Then raise the temperature to 160° C. and continue to heat until the pasty mass becomes pulverulent. Mix the crude product obtained with 3000 c.c. of water and 350 c.c. of hydrochloric acid (sp. gr. 1.12), filter, and dissolve the precipitate in a mixture of 700 c.c. of cold water and 200 c.c. of 10/N caustic soda. The filtered solution, when acidified with 390 c.c. of hydrochloric acid (sp. gr. 1.12), yields oxalyl-p-arsanilic acid in the form of a fine white precipitate (Bertheim, 1911). The latter is nitrated by dissolving 116 g. in 300 c.c. of concentrated sulphuric acid, keeping the temperature down to 15 to 20° C. and adding drop by drop a mixture consisting of 26 c.c. of concentrated nitric acid (sp. gr. 1.4) and 26 c.c. of concentrated sulphuric acid. After nitration has been completed, the mixture is stirred for ½ hour longer, 1500 c.c. of water are added, and the

Aniline Method No. 2.

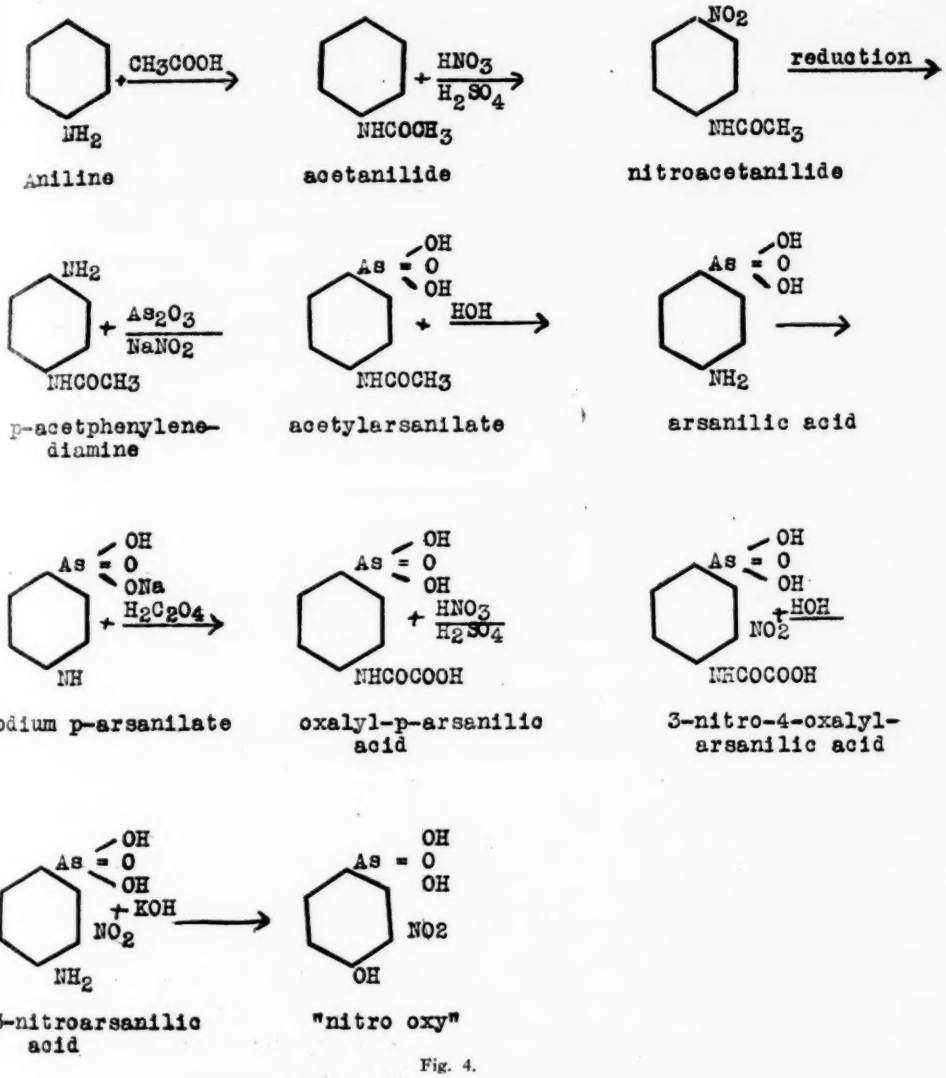


Fig. 4.

whole heated to boiling under a reflux condenser for 1 hour. On cooling, 3-nitro-4-aminophenylarsinic acid separates out in the form of long yellow silky needles (Bertheim, 1911). To obtain "nitro oxy," dissolve 500 g. of the nitro-compound in aqueous potassium hydroxide (500 g. KOH in 1500 c.c. H_2O), and heat the solution at 80° to 90° C. until the mixture becomes nearly solid. Ice-cold water (2000 c.c.) and hydrochloric acid are added successively, the precipitate dissolved in hot water, and the filtered solution treated with sodium acetate (1 mol.) and animal charcoal. After this treatment, the mixture is again filtered and the filtrate made acid with hydrochloric acid when "nitro oxy" separates either in yellow rhombohedral plates or in tufts of almost colorless needles.

The formulas in Fig. 4 show the course of the reactions described above: "Nitro oxy" is dimorphous in character, crystallizing in the form of yellow rhombohedral plates or tufts of almost colorless needles. It is also probable that the compound exhibits a chromoisomerism, in which there is a labile and a stable form. The work of A. Hantzsch (1910) with analogous compounds is suggestive in this connection. The substance is only slightly soluble in cold water or mineral acids, but is quite soluble in hot water forming a yellow colored solution. The color disappears almost completely on the addition of mineral acids. It dissolves readily in methyl and ethyl alcohol, glacial and 50 per cent acetic acid, but is insoluble in ether and acetic ester. It unites with alkalies forming salts which are soluble in water. The mono-sodium salt is bright yellow in color and gives an acid reaction; the di-sodium salt also gives an acid reaction but is orange colored; while the tri-sodium salt gives an alkaline reaction and occurs as either orange or red colored crystals. "Nitro oxy" readily breaks down in alkaline solution yielding o-nitrophenol and oxides of arsenic.

The "nitro oxy" used in the preparation of arsphenamine should be the product described by Bertheim. The arsenic should be in position one and the nitro-group in position three in the benzene ring. Any other arrangement of these groups, as in the other isomers, will yield a final product which will not be identical with the original salvarsan. Furthermore, the "nitro oxy" employed should be a *pure* product as the presence of impurities is one of the great sources of trouble in attempting to obtain an end product of good quality. Impurities, if present, interfere with crystallization and adhere to all products even to the final stages of the process. It has been argued by some that the removal of these impurities is unnecessary at this time inasmuch as still others are formed in the process of reducing the "nitro oxy." Experience, however, has shown that it is advisable to remove as many impurities as possible before the process of reduction is begun.

Preparation of Arsphenamine Base.—"Nitro oxy" is reduced by a number of reagents, e.g., sodium amalgam, sulphurous acid with hydriodic acid as a catalyst, phenylhydrazine, thionyl chloride, phosphorous trichloride, stannous chloride, certain ferrous compounds, phosphorous acid, hypophosphorous acid, sodium hydrosulphite, et cetera. Phosphorous and hypophosphorous acids attack only the arsenical group, while sodium hydrosulphite reduces both the nitro and arsenical groups. In the production of arsphenamine base, sodium hydrosulphite and certain combinations of the other reagents mentioned have been employed.

Two methods for the reduction of "nitro oxy" to arsphenamine base on a commercial scale are in use. They are known as the progressive and direct methods, respectively. When the first procedure is followed, "nitro oxy" is reduced progressively to 3-amino-4-hydroxyphenylarsinic acid, 3-amino-4-hydroxyphenyl-arsenous oxide and arsphenamine base or a similar series of compounds, whereas complete reduction is effected in one operation by the direct method. In reality, however, both procedures are continuous since the products of reduction obtained by the first method are not isolated at the

different stages enumerated. Reduction by the progressive method may be accomplished as follows:

Sodium amalgam (840 g. of 4 per cent Na) is added to 31.6 g. of "nitro oxy" in 600 c.c. of methyl alcohol at a temperature of 60° to 70° C. About ⅚ of the alcohol are distilled off, the residue extracted with 120 c.c. of water, the supernatant liquid decanted to remove the mercury, and the solution acidified with 150 c.c. of hydrochloric acid (sp. gr. 1.19). After 12 hours, the liquid is filtered, the filtrate boiled with animal charcoal and the clear solution treated with 52 c.c. of 10/N caustic soda. This completes the first stage, and the product obtained is crystalline 3-amino-4-hydroxyphenylarsinic acid (Ehrlich and Bertheim, 1912).

The second stage in the process consists in reducing the arsinic acid group of 3-amino-4-hydroxyphenylarsinic acid to arsenous oxide. This may be accomplished by saturating with sulphur dioxide a solution prepared by dissolving 230 g. of the arsinic acid, obtained as described above, in 2000 c.c. of water and 1000 c.c. of 2/N sulphuric acid to which has been added 50 g. of potassium iodide dissolved in 50 c.c. of water (Ehrlich and Bertheim, 1912).

The third stage, or reduction to arsphenamine base, may be conducted in the following manner. Sodium amalgam (28.8 g. of 4 per cent Na) is added to 30 c.c. of water and 32 c.c. of 2/N acetic acid containing 4.98 g. of the 3-amino-4-hydroxyphenylarsenous oxide. When the amalgam is used up, a further addition is made of 25 c.c. of 2/N acetic acid and 28.8 g. of sodium amalgam. This treatment is again repeated, when reduction is usually complete as a test with sodium hydrosulphite will show. The yellow precipitate formed is arsphenamine base.

Direct reduction of "nitro oxy" to arsphenamine base is effected by means of sodium hydrosulphite, or sodium hydrosulphite in combination with magnesium chloride. The presence of magnesium chloride is thought by some to prevent overreduction and to assist in the removal of sulphur compounds. Our own experience, however, has shown that equally good arsphenamine can be obtained with the use of sodium hydrosulphite alone. Inasmuch as sodium hydrosulphite is a comparatively little known reagent, a description of the compound is given at this point.

Sodium hydrosulphite, or "hydrosulphite" as it is commonly spoken of in the industry, is said to correspond in composition to the formula $Na_2S_2O_4$. It is a white or grayish-white solid containing about 2 per cent of zinc in addition to other impurities. It dissolves in water with the evolution of SO_2. It is evident that a part of its value as a reducing agent is due to this latter property, but its chief value in connection with the reduction of "nitro oxy" is claimed to be due to a change in the valency of the sulphur under the conditions of the reaction. Before a reduction can be successfully carried out with this compound, its reducing power must first be determined. This is usually accomplished by titration with an indigo solution.

The preparation of arsphenamine base, using "hydrosulphite" alone as the reducing agent, is conducted as follows: About 197 g. of "nitro oxy,"

accurately weighed, are dissolved in 4500 c.c. of water warmed to 55° C. to which sufficient caustic soda to form the disodium salt has been added. If the "nitro oxy" has been properly prepared, a clear yellow solution will result. Approximately 13 liters of water and ice are placed in a porcelain or glass lined vessel of at least 30 liters capacity and a weighed amount (usually about 2950 g.) of "hydrosulphite," calculated on the reducing power, is slowly added. If the resulting solution is dark in color, it should be discarded as it will not yield a satisfactory reduction product. The solution of "nitro oxy" is slowly poured into the solution of "hydrosulphite" with constant stirring. When this operation has been completed, the temperature of the mixture will be between 10° and 14° C. At this point, the temperature is raised about 2° per minute up to 30° C., and then less rapidly until 68° C. is reached, the stirring being continued throughout the operation. When a temperature of 33° to 35° C. is reached, the arsphenamine base begins to precipitate and continues to come down until reduction is completed. This usually requires that heating and stirring be continued for 1½ to 2 hours after the temperature has been brought up to 68° C. The stage at which reduction is complete is determined by removing a sample of the mixture, filtering and warming. Reduction is complete when the filtrate remains clear. When this point is reached, heating is discontinued and the precipitate allowed to settle during 1 hour. The supernatant liquid is then siphoned off and the yellow precipitate is collected on force filters of suitable size. Up to this stage, the precipitated base has been protected from the oxidizing action of the air by the sulphur dioxide liberated in the process of reduction. This protection is now continued by working in an atmosphere of carbon dioxide or other inert gas. The precipitate on the filter is sucked nearly dry and then washed with distilled water until the washings no longer give an acid reaction. It is then again sucked nearly dry when it is ready for conversion into the hydrochloride (Ehrlich and Bertheim, 1912, and others).

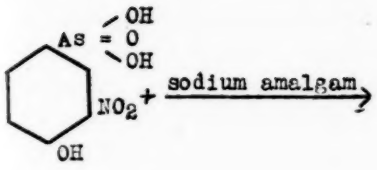
The preparation of arsphenamine base by this method requires careful supervision by an experienced worker. Slight variations in temperature and the masses of the reacting substances may give rise to the formation of by-products which are considered by some as possible causes for undue toxicity. These by-products may be either sulphur derivatives or compounds of the arsine or arsenoxide type.

The free base is a yellow powder soluble in dilute hydrochloric acid and aqueous caustic alkalies, slightly soluble in alcohol, and insoluble in water and ether. It is precipitated from alkaline solutions by acetic acid.

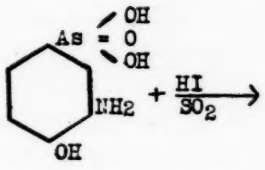
The formulas (Fig. 5) explain the reaction involved in these methods of reduction.

Preparation of the Dihydrochloride from Arsphenamine Base.—The free base obtained as described above is dried to the proper degree, mixed with 1700 c.c. of methyl alcohol, and the calculated quantity of methyl-alcoholic hydrochloric acid (0.75 mol. HCl) is added. The solution is filtered and the dihydrochloride precipitated by pouring the solution into absolute ether with

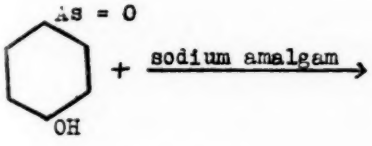
Progressive Reduction.



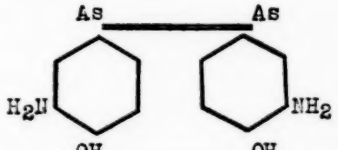
"nitro oxy"



3-amino-4-hydroxy-phenylarsinic acid ("amino oxy")

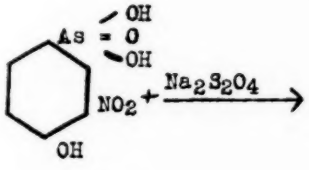


3-amino-4-hydroxy-phenylarsenious oxide

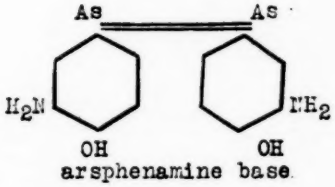


3:3-diamino-4:4-dihydroxy-arsenobenzene (arsphenamine base)

Direct Reduction.



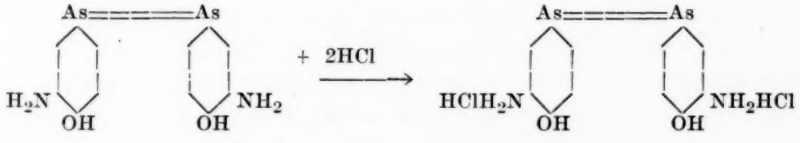
"nitro oxy"



arsphenamine base

Fig. 5.

vigorous stirring. The precipitate is collected on a force filter, thoroughly washed with ether and removed to vacuum chambers, where it is kept for 4 or 5 days before it is put into ampoules. The product thus obtained is arsphenamine, or 3:3′-diamino-4:4′-dihydroxyarsenobenzene containing an amount of volatile equivalent to approximately $2H_2O$.



In carrying out the above operation, trouble is sometimes caused by the precipitated dihydrochloride sticking to the sides of the vessel. This is due, either to the fact that the base was not dried to a sufficient degree, or to the

presence of impurities resulting from the improper reduction of the "nitro oxy." Another source of trouble is the formation of small cracks in the material when on the filter. A partial oxidation of the material may result from the sucking of air through these fissures.

Arsphenamine is a yellow powder permanent when dry and preserved in containers with an inert gas. It is readily soluble in cold water, methyl alcohol, glycerol, ethylene glycol and dilute hydrochloric acid, slightly soluble in ethyl alcohol. The aqueous solution is acid to litmus and turns Congo-red to violet-blue. Acid aqueous solutions decompose slowly on exposure to air, changing from yellow to red or brown in color. Alkaline solutions decompose comparatively rapidly under similar conditions becoming dark brown in color. Decomposition under these conditions probably results in oxidation with the complete breakdown of the molecule as shown by the formulas in Fig. 6.

The free base is precipitated from aqueous solutions of arsphenamine by alkali hydroxides and carbonates. In the case of alkali hydroxides, it is first

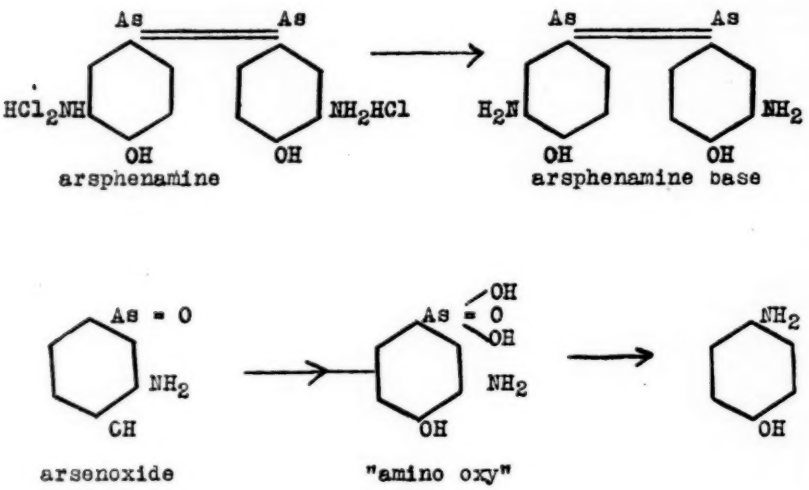


Fig. 6.

precipitated and then redissolved with the formation of the mono- or disodium salts, depending on the quantity of alkali added. Carbon dioxide precipitates the free base from solutions of the latter. For further descriptions of the properties of this compound, see Myers and DuMez (1918).

Packing.—The dry cakes of arsphenamine are carefully powdered in large mortars and the pulverized material passed through a fine-meshed sieve. The sifted powder is then returned to the vacuum chambers to allow any ether which may have been occluded in the caked material to evaporate. It is then divided into small portions, weighed quickly and the correct amount poured into each ampoule. The filled ampoules are placed in a vacuum chamber and the air exhausted, after which carbon dioxide or some other inert gas is allowed to flow in until atmospheric pressure is again reached. After this

operation has been completed, the ampoules are immediately sealed and labeled, the lot number being placed on each ampoule.

Due to atmospheric conditions at the time of filling, the inner surface of the ampoules may appear slightly cloudy. This condition, however, is no indication that the contents are not of good quality or have deteriorated. The factor which influences the quality of the product to the greatest extent at this stage is the care which is taken to prevent undue exposure to the air.

There is no relation between the color of the product and its toxicity. The same applies to the relation between solubility and toxicity. Products which are in true solution show no relation to toxicity, i.e., the ease of solubility is no criterion as to the toxic effects.

(*To be concluded in January issue.*)

LABORATORY METHODS

A NOTE ON THE ESTIMATION OF BLOOD CHLORIDES IN TUNGSTIC ACID FILTRATES

By John B. Rieger, M.D., Detroit, Mich.

IN OCTOBER, 1920, it was stated by the present author that the Volhard titration of chloride could be directly applied to the tungstic acid filtrate obtained in the Folin-Wu system of blood analysis, provided the reagents be free of chloride. The method presented[1] has lately been criticized on theoretic grounds by Whitehorn[2] who proposes that the excess of silver nitrate be titrated without removal of the silver chloride precipitate, as in the older method.

As a matter of fact neither centrifugalization nor filtration of the precipitated chloride need result in a loss of silver. The purine derivatives are never precipitated under the conditions prescribed and silver tungstate only begins to settle out when the concentration of sodium tungstate in the filtrate reaches 50 mg. per 100 c.c. Filter papers that contain chloride must, of course, not be used. The Whatman No. 2 paper has produced positive errors as high as 4 per cent, while Nos. 40 and 44, respectively, have been found free of chloride. Check analyses on mixtures of pure sodium chloride and sodium tungstate, the latter in concentrations of 25 mg. per 100 c.c., have given results invariably within 1 per cent of the theoretic figure, using either filtration or centrifugalization to remove the silver chloride.

The solubility of tungstic acid in 50 per cent nitric acid is between 15-20 mg. per 100 c.c., and is not appreciably less in the 66 per cent acid used by Whitehorn in his chloride test. Failure to obtain a turbidity with an equal volume of concentrated nitric acid is therefore entirely safe as a test for harmful amounts of tungstic acid in the filtrate. A positive test usually means incomplete precipitation of proteins and this interferes with the subsequent titration of the excess silver.

It is true that sodium tungstate can now be obtained free of chloride, but it is still advisable to purify it to get rid of the excess of alkali. In this way an accurate 10 per cent solution may be prepared and the juggling with acid to secure a complete precipitation of protein is obviated. Purification by alcohol precipitation as recommended by Folin is no doubt the better way, but pure alcohol cannot readily be obtained by workers outside of governmental or educational institutions and its recovery by distillation is prohibited, so that many are compelled to use an alternative procedure, such as described by the present author.

Sodium hydroxide preparations do not "always" contain large amounts of chloride, as stated by Whitehorn. That made from the metal theoretically cannot and actually does not contain it, and that purified by alcohol contains only harmless traces. The technical sodium hydroxide which Folin recommends for nitrogen determinations of course always carries large amounts of chlorides and must not be used.

In respect of the manner of precipitating the proteins in samples of whole blood, it has been the author's experience that addition of the acid before dilution with water is an effective procedure and actual analyses have shown that the distribution of chloride is in no wise changed, if the blood be allowed to stand one hour as directed. It will repay one to do this if a nonprotein nitrogen determination be desired on the filtrate, because occasionally when filtered immediately, the liquid will foam during the digestion, making the results worthless and the specimen of blood cannot always be replaced.

It may also be mentioned that oxalate is preferred to citrate as an anticoagulant because the latter interferes with the alkalimetry of the specimen should that be desired. The light green color obtained through its interaction with the ferric alum causes the pink color of the ferric sulphocyanate to appear brown but it does not interfere with the endpoint in the concentration advised.

The filtrates will, of course, always contain more or less tungstic acid. This serves a useful purpose by inhibiting the coagulation of the precipitated chloride until the mixture has been diluted to the mark and vigorously shaken. Obviously, any silver nitrate occluded under these conditions cannot influence the results of the titration, since the latter is carried out on aliquots. Silver chloride will coagulate upon shaking without addition of nitric acid and its failure to do so implies the presence of too much tungstic acid. The addition of nitric acid overcomes this but forms curds which can occlude more or less silver nitrate, and since Whitehorn does not employ aliquots of the filtrate, his method would theoretically be more likely to give high results than the method of the present author.

As evidence of the accuracy of his procedure, Whitehorn publishes a series of check determinations of chlorine in plasma, showing some of the results in agreement to within 0.01 mg., per c.c. It is difficult to understand how such close checks can be obtained unless a burette delivering one drop to 0.01 c.c., be used, since the formula given on p. 451 (l.c.) reads 5.00 – titer in c.c. = mgs. of chlorine per c.c. of blood or plasma. Using the ordinary laboratory burette, delivering 0.05 c.c. to a drop any deviation from the theoretic figure must be at least $\pm$ 0.05 mg. per c.c.

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THE METHYL ALCOHOL IN ARSPHENAMINE (SALVARSAN)*

By Philip Adolph Kober, B.S., Nepera Park, N. Y.

SINCE Ehrlich's work on salvarsan, it was generally assumed that, since the arsenic content of the drug is 31.6 per cent and not 34.4 per cent as it ought to be for the pure dry drug, Ehrlich's preparation and those preparations made according to his formula or method, have two molecules of water of crystallization which would account for an arsenic content of 31.6 per cent as it is found on analysis. In 1918[1] while working at the New York State Health Laboratories, I tried to show that there really was no valid reason for assuming that there were two molecules of water in the drug, but that all the evidence pointed to the presence of a molecule of methyl alcohol, free or combined, in the finished marketed drug. In the same paper I described a method for preparing a methyl-alcohol-free arsphenamine from aqueous solutions entirely.

Since then, from various sources, this work and the conclusion drawn from it has been disputed, and findings and conclusions of other investigators have been so different that we ought if possible account for these differences. That is the object of this paper. I also wish to discuss briefly the toxicologic effect of the methyl alcohol in the drug, and the properties of a methyl-alcohol-free arsphenamine preparation.

The first criticism encountered was that a methyl-alcohol-free arsphenamine was not a real arsphenamine since it differed from the Ehrlich product in constitution and somewhat in physical properties. The assumption underlying this criticism was that Ehrlich made all of his fundamental work on spirochetes, *in vitro* and *vivo*, with arsphenamine, which known as No. "606" is the *dihydrochloride of the salvarsan base*, the curative principle of all arsphenamine and neoarsphenamine preparations. Therefore it was concluded that a methyl-alcohol-free arsphenamine meant going into the field with a new drug of unknown therapeutic power!

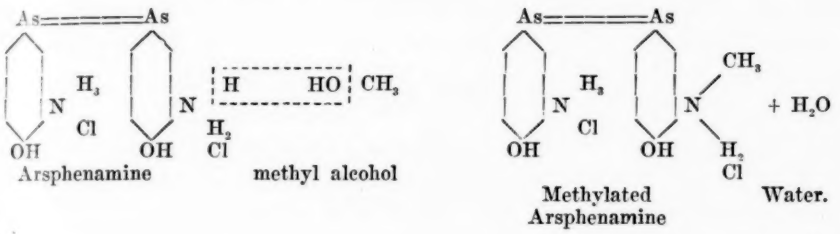
The fact is that Ehrlich[2] did not make his fundamental experiments with "606" but with No. 592, which is the salvarsan base, and therefore the arsphenamine preparation which yields upon neutralization the pure and original base, is a true Ehrlich preparation, like upon which his fundamental work was based. The importance of a pure arsphenamine base is also discussed in more detail below.

The second criticism was that after all there was no methyl alcohol present in Ehrlich's preparation or in preparations made according to his method. Meyer[3] from the laboratories of the H. A. Metz, Inc., reported that he had dried the Metz preparation on a large scale and found appreciable water,

*From the Laboratories of the Kober Chemical Company, Inc., Nepera Park, N. Y.

and traces only of hydrochloric acid and methyl alcohol. Meyer was asked at the meeting when this statement of his was made, how he determined his methyl alcohol and the writer was informed that it was a trade secret. Even from the meagre data available it was evident that the solvents in the drug, after having dissolved the drug in water, were not distilled, but the drug dried more than usual and an attempt made to determine the volatile products.

The Metz product is claimed to be identical with the German and Ehrlich salvarsan and therefore made with methyl alcohol and ether. The only way for their product to give off water is by the methyl alcohol combining with arsphenamine forming methylated arsphenamine, and water produced as a result of the reaction with methyl alcohol, in accordance with the following:



Fargher[4] and Pyman of the Burroughs, Wellcome & Co. Laboratories, London, also came to the conclusion that salvarsan contains no combined or free methyl alcohol. These authors made two combustion analyses on a sample of arsphenamine and found no increase in carbon content that would be in harmony with methyl alcohol present in the drug. However the hydrochloric acid content of these preparations was about 50 per cent below what arsphenamine is supposed to contain so that these two combustion analyses can have very little if any weight. These authors then dissolved some arsphenamine and distilled the resulting solution and got no more than traces of methyl alcohol. Whether here the methyl alcohol had been attached to the arsphenamine molecule as in reaction No. I, due to excessive drying and therefore methylation, cannot be proven here, but if the samples taken for combustion analysis are any criterion that is just what was done. These authors admit that when the drug is precipitated by means of acetone, even though it is no solvent for arsphenamine such as methyl alcohol is, the finished drug contains a whole molecule of acetone.

That arsphenamine made according to Ehrlich's directions does contain methyl alcohol is supported by the following facts:

(1) That Ehrlich[5] himself found methyl alcohol by qualitative as well as by quantitative methods in one of his batches.

(2) That Rieger[6] found a "strong Mulliken and Scudder" methyl alcohol "test on samples of German salvarsan, American salvarsan and Diarsonal."

(3) I have repeatedly found methyl alcohol and other organic solvents, in arsphenamine made by Ehrlich's method and even after a reprecipitation with hydrochloric acid, on distillation from an aqueous solution obtained a methyl alcohol test.

(4) Raiziss from the Dermatological Research Laboratories has admitted that arsphenamine contains methyl alcohol[7] and in a recent article[8] gives results which confirm my finding of alcohol in neoarsphenamine.[9]

(5) The drug can hardly have any water in it, when it is precipitated from absolute methyl alcohol and anhydrous ether, and must contain methyl alcohol or some other impurity to account for its low arsenic content.

Considering that all positive evidence is in support of methyl alcohol being present in arsphenamine made according to Ehrlich's method, and that negative evidence is accounted for by faulty or unsuited technic, there seems no doubt, then, that Ehrlich's salvarsan was contaminated with one molecule of methyl alcohol, either free or combined, depending upon whether the solvents and material were extremely anhydrous and whether the product was dried much or not.

The Toxicological Effect of the Methyl Alcohol

The question arises what is the effect of the presence of a molecule of methyl alcohol. The amount of methyl alcohol is not large, being about 7 per cent or about 42 milligrams in a dose of 0.6 gram. What the direct toxicological effect of 42 milligrams of methyl alcohol is, when injected intravenously into the body, in the presence of arsphenamine, is not definitely known. Unless large amounts of the drug containing free methyl alcohol are frequently injected, no direct effect is likely to be felt, unless it is administered in cases of complications.

When the methyl alcohol is combined to the arsphenamine the effect is likely to increase the toxicity. Ehrlich and his collaborators[10] made methyl derivatives and found that they were very toxic and therapeutically not effective. Therefore the presence of combined methyl alcohol is apt to increase the toxicity and lower its therapeutic value.

Rieger[11] in his published work came to the following conclusion: "Commercial arsphenamine may contain an arseniurated methyl compound which decomposes either in the ampule or in solution, with liberation of arsenous oxide or a cacodyl-like substance. Some preparations betray the presence of the arsine by their garlic-like odor when dissolved; others develop it only after having stood in solution for hours. According to the amount that may have accumulated, the dosage and the idiosyncrasy of the patient, a reaction marked by fall in blood pressure, dyspnea and cyanosis may occur. Once the drug has been injected, the occurrence of subacute or chronic arsenic poisoning is determined by the margin that exists or may be made to exist between its elimination and its reduction by the tissues. There is evidence that this reduction to metallic arsenic occurs too readily for safety with present commercial preparations of arsphenamine."

Furthermore the technic involving methyl alcohol and ether is from a chemical point of view unsuited for treating a very sensitive and highly reduced substance like arsphenamine. Alcohol and especially ether with its peroxides are likely to act as organic catalysts, causing oxidation and other

changes in the constitution of the drug. This indirect effect of the presence of the methyl alcohol is in harmony with the toxicity of the drug, as found on the market. The German drug seems to have a tolerated dose of 60 mg. per kilo on white rats, the American product as it first appeared on the market had about the same toxicity, and owing to increased skill in handling the product, especially in keeping the air away and working at low temperatures, the toxicity of the American product has gradually decreased so that 100 mg. per kilo is the Standard of the U. S. P. H. Service today. But the fact is well known that manufacturers find from time to time that their product is too toxic to be used, without, however, knowing the direct cause of the trouble. This seems never to be the case if the arsphenamine is prepared without the use of methyl alcohol and ether. I[11] found that I had no difficulty with the hydrochloric acid method of preparing arsphenamines that were in every instance above the 100 mg. per kilo standard. Christiansen[12] working in Professor Reid Hunt's laboratory found that with the hydrochloric acid method he could get arsphenamine which averaged 140 mg./kilo never being below 120 mg./kilo for albino rats. Some batches went as high as 160 mg./kilo. Trying the method for the first time M. Lewis I. Nurenberg, of the Massachusetts Health Department Laboratory found that he could get 130 to 140 mg./kilo toleration from reworking old and toxic batches.

The Properties of a Methyl-Alcohol-Free Arsphenamine

It was found that when arsphenamine was made especially pure and not excessively dried, it did not dissolve in cold water quite so readily, owing to its having an increased tendency to gelatinize when first moistened with water. Thus, Ehrlich's[13] first products dissolved slowly! The products first made by Raiziss[14] in the Dermatological Laboratories dissolved slowly, so that boiling water was called for in the printed directions accompanying the drug. The products made by me with the hydrochloric acid method[15] also dissolved slowly. Fagher and Pyman[16] also found that their purest arsphenamine, made for scientific study was of slow solubility. Christiansen's purest products, some with a tolerated dose of 60 per cent above the Government standard dissolved slowly. In short, arsphenamine made pure by three different methods reveals a drug with a tendency to produce a slowly dissolving gel in cold water and rapidly dissolving gel in hot or warm water, so that we are forced to the conclusion that the slow solubility in cold water is a characteristic of the pure arsphenamine. Therefore we should not change the chemical composition of the drug for the purpose of hastening solution. The gain of a minute or two of time in dissolving is not commensurate with the loss of chemical constitution of the drug and its resultant danger to the host and loss of therapeutic power.

After the arsphenamine has been dissolved in water, it must be properly and quickly neutralized, so that air is not allowed to act long on the alkalinized solution, as Roth[17] has shown that exposure of the alkaline solution to the air results in an increase of toxicity of the drug.

SUMMARY

The properties of a pure arsphenamine are:

(1) It gelatinizes in cold water.

(2) Its gel dissolves in warm water.

(3) Its color is a light straw in solution.

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[14]Printed circulars of Dermatological Laboratories.
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[17]Public Health Reports, U.S.P.H.S., xxxv, No. 38, pp. 2205-2210.

BLOOD COUNTS WITH OXALATED BLOOD*

By Nancy Yarbrough, Charlotte, N. C.

IT IS often very difficult for the physician who visits out-of-town patients to find the ways and means of having his blood counts made. In order to help solve this problem we have worked out a plan whereby the blood can be procured by the visiting doctor and brought back to the home base for examination. The method of procedure is very simple and one that every practicing physician can carry out.

MATERIALS

1. A sterile 5 c.c. syringe.

2. A sterile tube containing dried potassium oxalate. Potassium oxalate prevents clotting and four drops of a 20 per cent solution, dried in the hot air sterilizer, will preserve 30 c.c. of blood.

METHOD

Draw blood from the vein and immediately place in the oxalate tube and shake thoroughly for one minute: cork securely and take to the laboratory for counting.

In this laboratory blood was taken as described above and at the same time counts were made on blood taken from the ear or finger of the same patient.

*From the Laboratory of the New Charlotte Sanatorium.

Counts on the oxalated blood were approximately identical with those taken from the finger. Complete blood counts can thus be made satisfactorily on oxalated blood. Below a few exact figures are cited.

CASE 1. Leucocyte count on fresh blood, 9,400, on oxalated blood, 8,700.

CASE 2.

	Fresh blood	*Oxalated blood*
Leucocytes	18,000	17,700
Red cells	3,328,000	3,464,000
Hemoglobin	45 %	45 %

CASE 3.

	Fresh blood	*Oxalated blood*
Leucocytes	7,500	7,500
Red cells	4,352,000	4,072,000
Hemoglobin	90 %	90 %
Differential count (200 cells counted)		
Polynuclears	67 %	62.5 %
Mononuclears	28	32.5
Eosinophils	5	4.5
Basophils	0	0.5

From the above figures it can easily be seen how nearly identical are the counts on fresh and oxalated blood. Two people making counts on fresh blood would probably vary as much in their respective reports. Oxalated blood will keep at least three days, giving correct counts at the end of this time. The following precautions are necessary:

1. Blood must be forced immediately out of the syringe into the oxalated tube.

2. The blood must be shaken thoroughly for at least one minute. Any clotting whatsoever destroys the accuracy of the count.

3. The blood must again be shaken thoroughly before counts are made.

AN APPARATUS FOR THE ESTIMATION OF CATALASE*

BY WM. H. WELKER, A.C., PH.D., CHICAGO

SINCE catalase had been assumed to run parallel with oxydase, and consequently supposed to be an index of the oxidative activity in the organism or in various portions of the organism, it seemed desirable to try the effect *in vivo* of a reagent such as potassium cyanide, which according to the generally accepted view, diminishes oxidation.

At the time this work was begun, most of the investigators studying catalase had been doing the shaking by hand and some of them were not even using leveling bulbs in connection with the measuring tubes, and consequently were introducing large experimental errors. It seemed desirable to construct a machine by which the mixture of blood and hydrogen peroxide could be shaken uniformly and by which a series of determinations could be carried out at one time, with provision for reasonably accurate measurement of the gas liberated.

*From the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois.

The machine shown in Fig. 1 consists essentially of a support of three-eighth-inch pipe from which a board is suspended by four brass rods. The eccentric on the shaft of the driving wheel is connected with this suspended board by a rod having a flexible joint at the board end. The bottles used in the machine are 500 c.c. salt-mouth bottles, ground for glass stoppers. These bottles are supported on the board by means of copper cans fastened to the board, and are held rigidly in the cans by means of thermos bottle springs. Ordinary rubber stoppers proved unsatisfactory for closing the bottles. This difficulty was overcome by having made to order, some double length stoppers. These can be seated so firmly in the bottle necks that no gas leakage occurs. For measuring the liberated gas each of the bottles is connected with

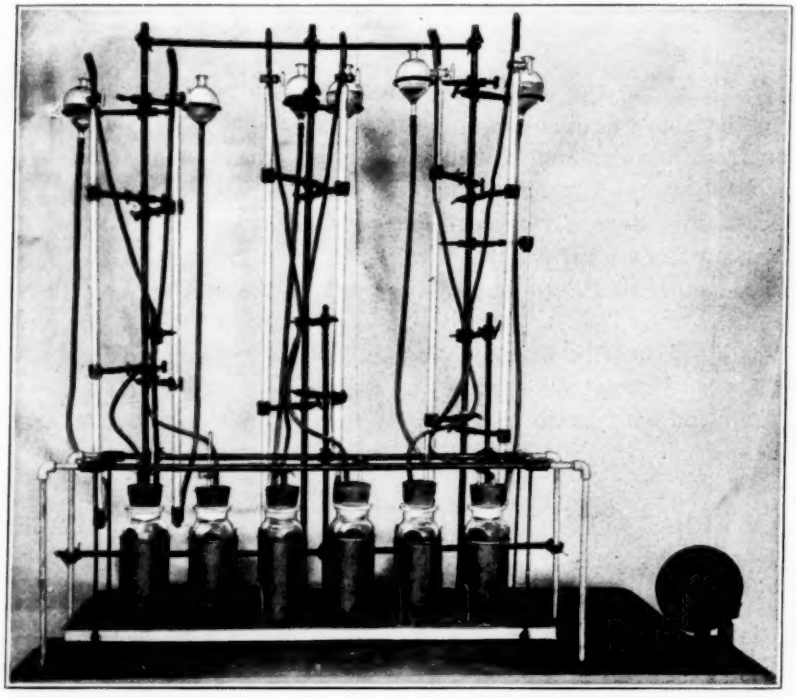

Fig. 1.

the top of a gas measuring tube by a rigid wall, rubber tube. To the bottom of the gas measuring tube is connected a leveling bulb. The six gas measuring tubes with their leveling bulbs are supported by a special stand built for the purpose. Aluminium caps from screw-cap vials are used as containers for the blood.

The mode of operation is as follows: 75 c.c. of diluted hydrogen peroxide (equal volumes of hydrogen peroxide and water) are measured into each of the bottles. The samples (usually .5 c.c.) of blood are then accurately measured into the aluminium caps. These are floated on the surface of the hydrogen peroxide by means of a pair of tongs, made by straightening out the curved ends of a pair of crucible tongs. The stoppers are then placed firmly

into the bottle necks. The stopcocks on the bottles are opened and the level of the liquid in the gas measuring tube adjusted so that the reading is zero. The stopcocks on the bottles are then closed and the shaking is commenced. In the work carried on with this machine, readings have been taken every five minutes, at atmospheric pressure.

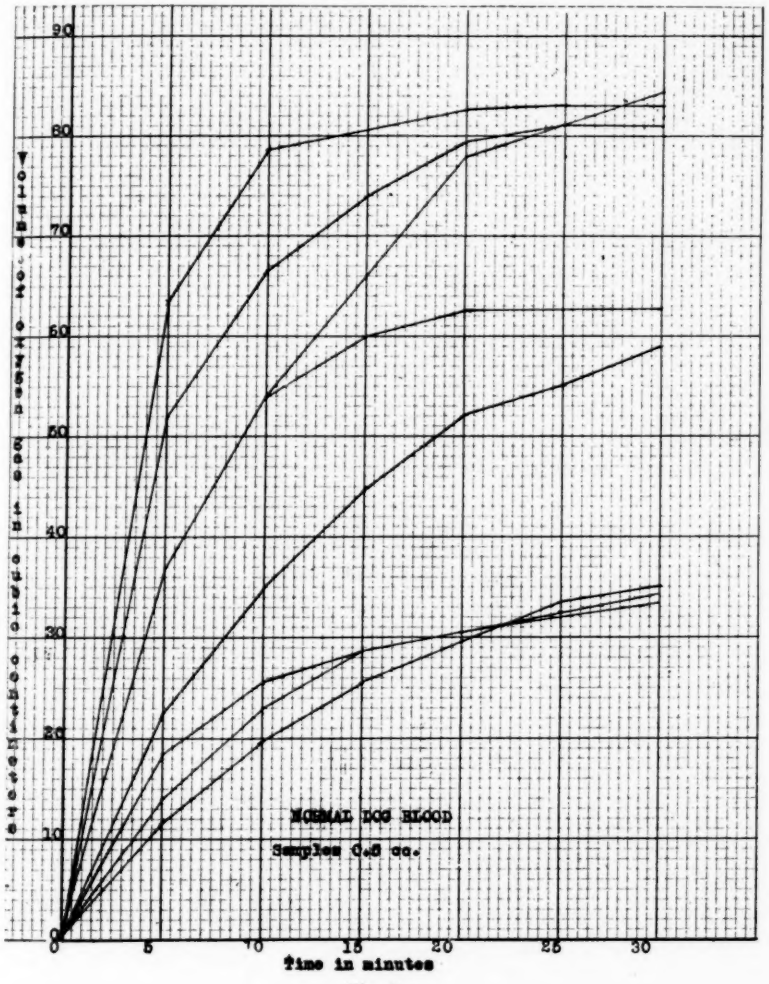

Fig. 2.

Fig. 2 shows a series of curves obtained from normal blood of dogs. These curves are based on results corrected for temperature, barometric pressure, and aqueous tension.

I am indebted to Dr. T. B. Magath for assistance in working out some of the details of the apparatus.

THE ESTIMATION OF INORGANIC PHOSPHORUS IN BLOOD PLASMA BY THE METHOD OF BELL AND DOISY*

By Burton A. Myers and Marian C. Shevky, San Francisco, Cal.

WE HAVE met with certain difficulties in the practical application of Bell and Doisy's[1] recently published method to the determination of inorganic phosphorus in blood plasma which we believe are worthy of note, because their removal may broaden the usefulness of a method which has the

Table I

Known Concentrations of Phosphorus Read Against a Standard Containing 0.5 Mg. P per 100 c.c.

Actual P concentration Mg. per 100 c.c.	P concentration found Mg. per 100 c.c.	Error %
2.00	1.73	−13.5
1.75	1.56	−10.9
1.50	1.35	−10.0
1.25	1.08	−14.0
1.00	0.85	−15.2
0.75	0.67	−10.7
0.25	0.25	± 0.0

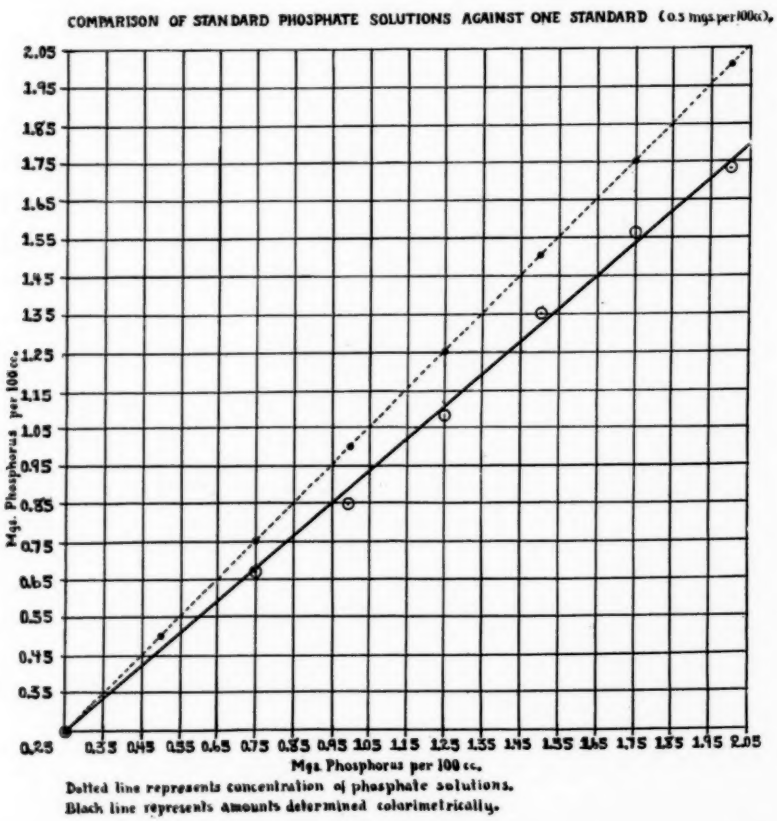

Fig. 1.

*From the Laboratory of the Division of Medicine, Stanford University Medical School, San Francisco, Calif.

great advantage of comparative simplicity. We were led to the study on account of a failure to recover phosphorus added to plasma.

A series of solutions of varying concentrations of acid potassium phosphate (KH_2PO_4) were prepared which corresponded to the range of concentration in which inorganic phosphorus is known to exist in protein-free plasma filtrates. The solution containing 0.5 mg. of phosphorus per 100 c.c., which is probably near the average phosphorus concentration of plasma filtrates, was taken as the standard. Results were obtained as shown in Table I and Figure 1.

The only solution correctly determined was the one which was weaker than the standard, and all the others showed considerable minus errors.

TABLE II

KNOWN CONCENTRATIONS OF PHOSPHORUS READ AGAINST A STANDARD CONTAINING 0.5 MG. P PER 100 C.C. AFTER DILUTING EACH WITH WATER IN THE AMOUNT THEORETICALLY REQUIRED TO MAKE THE COLORS EQUAL

ACTUAL P CONCENTRATION MG. PER 100 C.C.	P CONCENTRATION FOUND MG. PER 100 C.C.	ERROR %
2.00	1.38	−36.0
1.75	1.12	−31.5
1.50	1.02	−32.0
1.25	0.87	−30.5
1.00	0.73	−26.7
0.75	0.61	−19.5

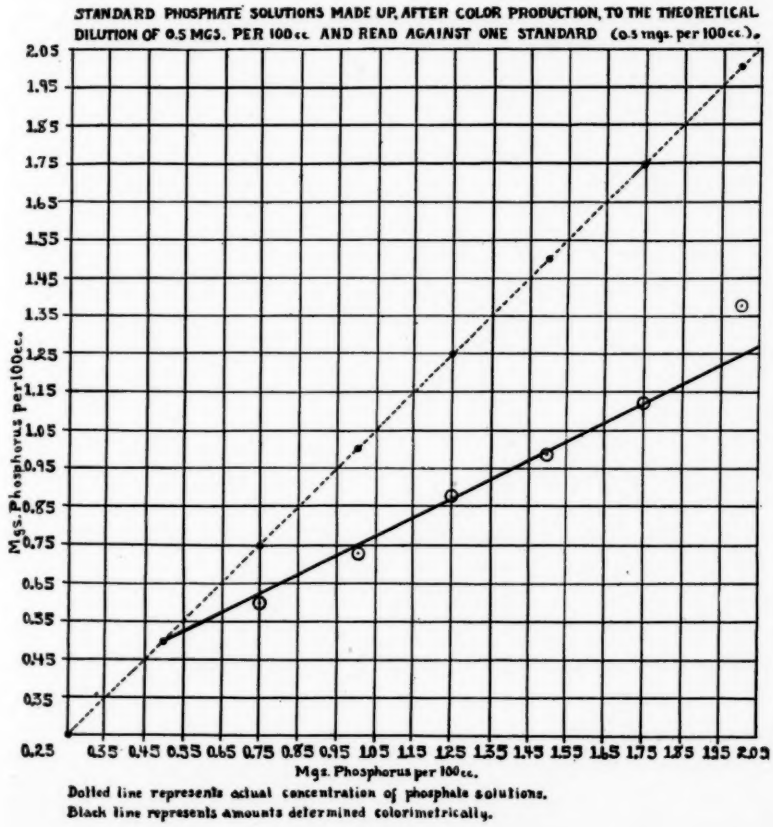
STANDARD PHOSPHATE SOLUTIONS MADE UP, AFTER COLOR PRODUCTION, TO THE THEORETICAL DILUTION OF 0.5 MGS. PER 100 cc. AND READ AGAINST ONE STANDARD (0.5 mgs. per 100 cc.).
Mgs. Phosphorus per 100cc.
Mgs. Phosphorus per 100 cc.
Dotted line represents actual concentration of phosphate solutions.
Black line represents amounts determined colorimetrically.

Fig. 2.

In order to obviate the difficulty of comparing colors varying considerably in density, the same procedure was repeated except that after the development of the blue color all the solutions were diluted with water in amounts which should theoretically have made all the colors equal. The results are given in Table II and Fig. 2.

It is clear from these figures that the amount of the blue color which appears is not directly proportional to the amount of phosphorus actually present, and that it was this fact which accounted for our inability to recover all the phosphorus we added in our experiments with plasma.

Bell and Doisy state: "It may be necessary to use a stronger standard or less filtrate in bloods containing much phosphorus. The proper standard and amount of filtrate may be roughly estimated before the determination is made." But there are no data given as to the degree of permissible difference between the standard and the unknown, and it is clear that this is an essential point to establish before the method can have any practical application. It has been noted by Greenwald[2] that the phosphorus in the blood serum of normal persons may range from 1.0 to 7.0 mg. of phosphorus per 100 c.c. It is also possible in normal individuals to increase the concentration of phosphorus beyond these limits.[3] Marriot and Howland[4] found a great increase in the inorganic phosphates in nephritis with acidosis, extending from 8.0 to 23.0 mg. of phosphorus per 100 c.c. of blood serum.

We have found that known phosphate solutions could be estimated with a fair degree of accuracy when the concentration of the standard was not more than 0.25 mg. of phosphorus per 100 c.c. greater than the unknown.

TABLE III

KNOWN CONCENTRATIONS OF PHOSPHORUS READ AGAINST DIFFERENT STANDARDS EACH CONTAINING 0.25 MG. PHOSPHORUS MORE THAN THE CONCENTRATION OF PHOSPHORUS EXAMINED

STANDARD MG. PER 100 C.C.	ACTUAL PHOSPHORUS CONCENTRATIONS MG. PER 100 C.C.	PHOSPHORUS CONCENTRATION FOUND MG. PER 100 C.C.	ERROR %
2.00	1.75	1.79	+2.0
1.75	1.50	1.49	-0.5
1.50	1.25	1.24	-0.8
1.25	1.00	0.99	-0.6
1.00	0.75	0.72	-3.7
0.75	0.50	0.53	+5.0

Table III and Fig. 3 show that in working with unknown solutions, it will be necessary to have a series of standard solutions, so that a standard may be selected which contains not more than 0.25 mg. of phosphorus per 100 c.c. more than the solution of which the phosphorus content is to be determined.

Another difficulty arose in work with rabbit plasma. In some instances, but not in all, little or no color was produced when the usual amounts of molybdic acid and hydroquinone solutions were used. It was found, however, that in all cases the color appeared, when to 5 c.c. of the plasma filtrate (1:5 dilution), 1.5 c.c. of molybdic acid and 3 c.c of hydroquinone solution were added. Since this variation in the amount of reagents used had no effect on the color pro-

duction, either in phosphate solutions or in plasma, we believe that it is safer to adopt this modification. This nonproduction of color is not peculiar to rabbit plasma, for we have seen it occur with one human plasma, though never in beef serum.

A small series of determinations of inorganic phosphorus in filtrates from plasma and beef serum before and after the addition of known amounts of

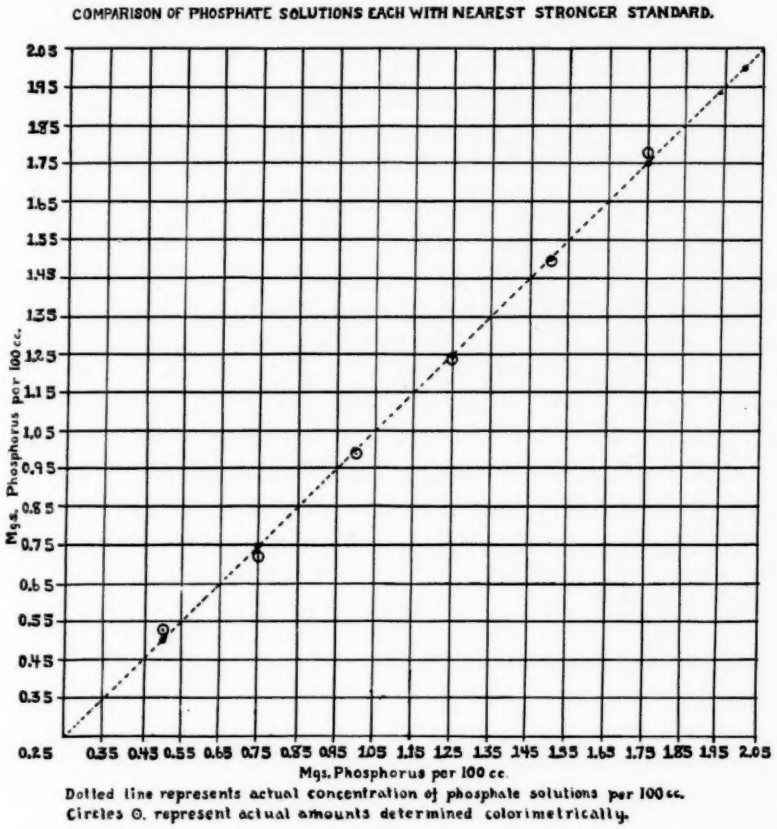
COMPARISON OF PHOSPHATE SOLUTIONS EACH WITH NEAREST STRONGER STANDARD.
Mgs. Phosphorus per 100 cc.
Mgs. Phosphorus per 100 cc.
Dotted line represents actual concentration of phosphate solutions per 100 cc.
Circles ⊙ represent actual amounts determined colorimetrically.

Fig. 3.

phosphorus showed that under these conditions the error of the method ranged from 2.3 per cent to 10.0 per cent with an average of 5.8 per cent. In every case the standard used contained phosphorus in an amount which did not exceed that in the unknown by more than 0.25 mg. of phosphorus per 100 c.c.

CONCLUSIONS

1. A series of standards must be prepared so that in each determination one may be selected which contains phosphorus in an amount which does not exceed that in the unknown by more than 0.25 mg. per 100 c.c.

2. In many rabbit plasmas and in some human plasmas larger amounts of molybdic acid and hydroquinone solutions must be added.

3. A small series of determinations of phosphorus in plasmas to which

known amounts of phosphorus were added indicated that the average error was about ± 6.0 per cent with a maximum of 10.0 per cent.

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NOTE ON THE DIFFERENTIAL STAINING OF THE GRANULES IN DIPHTHERIA AND OTHER BACILLI*

By Albert G. Nicholls, M.D., D.Sc., Halifax, N. S.

THE method of demonstrating the granules in bacteria described here was accidentally discovered by the writer in a moment of inadvertence. While staining a number of slides of bacteria by the Neisser double staining method and by Gram, the preparations became mixed. The error was discovered after certain of the slides had been treated with Neisser No. 1 and then with Gram's iodine solution, when examined at this stage it was found that granules in diphtheria bacilli were exceedingly well brought out, and it only required a little experimenting to produce a method of granule staining which had the merits of simplicity, certainty, and effectiveness. The procedure is as follows:

Stain films, fixed by heat, with Neisser's Staining Fluid No. 1. This consists of Methylene Blue, 1 gram; 96 per cent alcohol, 20 c.c.; glacial acetic acid, 50c.c.; and distilled water, 950 c.c. This mixture is allowed to remain on the films for thirty seconds. Wash in water. Apply Gram's iodine solution for ninety seconds. Wash in water. Apply watery solution of Saffranin T one-half per cent for thirty seconds. The granules appear black on a pink background. The picture is sharp, the contrast excellent. The method is applicable to any bacteria which contain granules, and shows up the granules in diphtheria bacilli exceptionally well.

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EDITORIALS

Arsphenamine and Neoarsphenamine Dosage

WHEN salvarsan was introduced into the medical world, the details of its administration had been already worked out. Ehrlich, after having chemically perfected the spirocheticide, had had its action tested on thousands of individuals in several of the best German clinics, so that when the treatment came into general use the experimental phase had been passed, and the dosage and manner of administration had already been worked out in detail. In this way unfortunate accidents in the hands of inexperienced physicians were to a great extent avoided.

Nevertheless there have been several important and valuable changes in the method of arsphenamine therapy during the last ten years. In the treatment of syphilis, it was soon discovered that the "therapia sterilisans magna," which had been satisfactorily employed in animals could not be reproduced in man. Our inability to destroy all of the spirochetes in the body with one massive intravenous medication has rendered it necessary to administer repeated doses. The question of dosage in the treatment of syphilis has therefore become extremely important.

Ehrlich in his original work on trypanosomes, recognized that in those cases not entirely cured by the first treatment, relapses were even more difficult to cure. Treatment which had been unsuccessful in a first attempt was usually unsuccessful on repetition. Repeated treatments, none of which cured entirely, were often found to have progressively less influence on the activity of the infecting parasite. A condition of "Festigkeit," increased tolerance or resistance to the action of the drug, had been produced.

The possible development of "drug-fastness" by the infecting agent in individuals undergoing antisyphilitic treatment, is now recognized as an important feature and a phenomenon to be avoided if possible. In the case of trypanosome infection Ehrlich found not only that the parasite became resistant to the chemical action of the drug, but also that the increased tolerance persisted after transplantation into another host. A strain resistant to atoxyl in the mouse maintained its high tolerance to the drug throughout 46 subsequent passages through rats. Eventually, however, the parasite tends to regain its original sensitiveness to the drug in question. The possible development of a race of spirochetes resistant to the action of arsphenamine, one for which we must find some new or stronger method of therapy, must be considered.

A contribution of considerable significance has been made recently by Bronfenbrenner and Schlesinger,[1] who found that minute doses of arsphenamine, instead of inhibiting the progress of the spirochetal infection, actually caused the disease to progress more rapidly and more extensively in the animals so treated than in other animals similarly infected but not treated at all. Bronfenbrenner and Noguchi[2] found some time ago that minute amounts of arsphenamine present in culture media stimulated the growth of spirochetes. Very minute quantities of arsphenamine appear therefore to be of less value than no treatment at all. The phenomenon appears analogous to that found in the treatment of cancer by the roentgen ray, where small doses stimulate the cells to proliferation while larger doses destroy. Bronfenbrenner and Schlesinger point out that this stimulative action of minute doses of arsenical compounds is not to be confused with the development of increased tolerance, but is characterized by an actual increase in the rapidity of multiplication of the parasites.

The dosage used by these investigators is of course extremely small and it does not follow from their report that the occasional small doses used therapeutically in the treatment of syphilis have a stimulating action. It is quite probable that even these small doses are sufficiently large that they inhibit multiplication. This is indicated by the customary diminution in the intensity of the positive Wassermann reaction.

Brown and Pearce[3] have shown in a recent article that insufficient treatment with arsphenamine or neoarsphenamine may alter the immunologic status in experimental animals to such an extent as to favor the development of a second or superimposed infection without a cure having been accomplished for the first. They inoculated rabbits, and after the development of the primary lesion, treated the animals with single massive doses of arsphenamine or neoarsphenamine. The primary lesions as a rule retrogressed and in some instances disappeared. Reinoculation, at a time when the drug was no longer present in the body in sufficient

amounts to produce an effect, and when the original primary lesion had practically disappeared, produced in most of the rabbits a second primary lesion at the site of reinoculation. In the majority of the animals the original infection had not been entirely destroyed and as the original parasites once again commenced to multiply, chancres developed both at the site of the original inoculation and the site of reinoculation.

It has long been held that individuals once infected with the spirochete of syphilis are immune to reinfection as long as the germ remains active in the body. Reinfection usually is assumed to be evidence that the patient had been cured of the former infection. The work of Brown and Pearce shows quite conclusively that experimentally at least, an individual may become reinfected with syphilis even though the original invaders are still living in his tissues. Similar phenomena have occasionally been reported in man.[4]

How frequently the factors discussed above will become of importance in the treatment of syphilis is uncertain. Certainly the standard doses in use in the large clinics have proved their worth. But that they are the ideal dosage does not follow. There is a tendency with many, to give relatively smaller doses of arsphenamine or neoarsphenamine than formerly. Past experience and recent laboratory observations appear to be of accord in indicating that the dosage in antisyphilitic treatment should be as high as safety permits.

Mehrtens[5] has recently reported on the administration of neoarsphenamine by the rectum. He does not prefer this method to intravenous therapy but suggests its use in cases where the latter is difficult or impossible, as in children or in individuals in whom it is impossible to enter the vein. Assuming the value of high arsenical concentration in the destruction of spirochetes one may conclude from Mehrten's tablets that rectal administration is not as desirable as intravenous treatment. Five minutes after the intravenous administration of 0.6 gm. of arsphenamine, arsenic is present in the blood in a concentration of 0.97 mg. per 100 c.c. By the end of an hour it has fallen to 0.077 mg. per 100 c.c. One hour after the administration of 4 gm. of neoarsphenamine by rectum, there is 0.0015 mg. of arsenic in each 100 c.c. of blood. Absorption from the rectum is slow and it requires 12 hours before the blood has attained a maximum concentration of 0.025 mg. per 100 c.c. From the 6th to the 14th hour after administration the concentration in the blood equals or exceeds that after intravenous administration but this amount is exceedingly small as compared with that to be found immediately after injection directly into the vein. It should also be borne in mind that the dose of neoarsphenamine by rectum is 4 gm. as contrasted with 0.6 gm. of arsphenamine by the vein. Mehrtens remarks that the actual quantities of arsenic present in the blood after administration by rectum is at all times very small.

He also observed the amount of arsenic excreted in the urine both after intravenous administration of 0.6 gm. of arsphenamine and following 4 gm. of neoarsphenamine by rectum. The amount of arsenic excreted in the urine during the first twelve hours was over three times greater following rectal administration than after intravenous administration. Very much the same ratio was found during the second 12 hour period. Even two days after

treatment, nearly three times as much arsenic was still appearing in the urine. Three days and a half after administration, arsenic was still present following both methods, but in rapidly diminishing amounts. Following a very much smaller dose intravenously, arsenic appears to have persisted in the urine nearly as long as after a massive dose through the rectum.

Mehrtens states that he observed no signs of toxicity after the large dose other than occasional attacks of vomiting, and in two cases, puffiness under the eyes. This latter observation might be associated with the added burden to the kidneys. Where there is renal impairment this method should probably be avoided.

Rectal treatment would theoretically appear inferior to intravenous therapy, first because the concentration of arsenic in the blood and probably also in the body tissues never becomes as high, and, second, because the kidneys are placed under an unusual strain. It should be repeated here that Mehrtens does not recommend this method as an improvement or even as a substitute for intravenous medication.

Adverse theoretical considerations are without weight in the presence of demonstrated clinical results. If new methods show better clinical and serologic results, they must be accepted as improvements. If these improvements are not logical in view of existing postulates, the postulates must be changed. So far, however, modifications of the usual intravenous technic have not proved their greater worth. Mehrtens recognizes the difficulty in estimating clinical results. He also points out that the subjects treated were chiefly old neurosyphilitics in whom improvement under any form of treatment is neither rapid nor satisfactory. He does believe, however, that serologic improvement was at least as rapid by the rectal method as following intravenous administration.

Minet[6] claims truly astonishing therapeutic results from the daily subcutaneous injection of 0.15 gm. of neoarsenobenzol. If the important factor in the care of syphilis is prolonged saturation rather than massive dosage, both the technic of Mehrtens and that of Minet become entirely logical. This possibility will be discussed shortly. Probably both factors play a part.

The standard intravenous dose of arsphenamine is 0.6 gm., that of neoarsphenamine 0.9 gm. These quantities appear to be sufficiently large for satisfactory therapeutic results and yet are not so large that they cause untoward results. They contain about equal amounts of arsenic and are supposed to have equal therapeutic value. The majority of critical observers have found, however, that there is a difference in the effectiveness of the two drugs when given in these doses. Most syphilologists have concluded that arsphenamine produces prompter results and is less liable to be followed by relapse. The popularity of the second preparation is due to the greater ease of administration, a factor which should play a less important rôle than it actually does. In nearly all of the armies, during the War, syphilis was treated with neoarsphenamine. This was undoubtedly due to the greater ease, and shorter time required for administration, thereby facilitating the treatment of larger numbers of individuals, and to the fact that considerably less apparatus was required. Rightly or wrongly this has given an official sanction to the use of neoarsphenamine.

Considerable experimental work has been done within recent years to determine if possible the comparative value of the two drugs. Schamberg, Kolmer and Raiziss,[7] working with trypanosomes, found that the trypanocidal activity of arsphenamine was 1.74 times that of neoarsphenamine. Castelli and others, working on trypanosomiasis, spirillosis, and rabbit syphilis reached similar conclusions, finding that the activity of the former was between 1.5 and 1.78 times that of the latter. If we are to accept these results we should conclude that therapeutically 0.6 gm. of arsphenamine is not equivalent to 0.9 gm. of neoarsphenamine, but rather to 1.05 gm. The therapeutic activity of 1.05 gm. of neoarsphenamine is equivalent to that of 0.6 gm. of arsphenamine.

The trypanocidal dose of arsphenamine (dosis therapeutica) is 4.564 times less than the highest tolerated dose, (dosis tolerata). On the other hand the therapeutic dose of neoarsphenamine is 6.35 times smaller than the highest tolerated dose. The latter is therefore, a safer drug, the margin of safety between the two doses being distinctly greater. This is true even if 1.05 gm. instead of 0.9 gm. of neoarsphenamine should be used.

Voegtlin and Smith[8] have studied the same problem from a different angle and have reached similar conclusions. They ascertained the minimum effective dose and the minimum lethal dose in trypanosome infected animals. The minimum effective dose is that dose which will destroy practically all trypanosomes in the circulating blood. It is not a curative dose. A fairly sharp threshold exists for this dose, below which the drug has comparatively little trypanocidal activity. A minimum concentration of the drug appears necessary for the destruction of the majority of parasites present in the blood stream. The dose, below which the drug fails to destroy the parasites, has been found to be fixed partly by the reaction between the drugs and the parasites, and partly by the rate at which the drug is absorbed by the tissues of the host. Subeffective doses cease to act, not when they have killed a certain number of parasites, but when absorption by the host has lowered the concentration below the threshold. Voegtlin and Smith believe that a relapse can be avoided only by giving considerably more than the minimum effective dose. The question both with arsphenamine and with neoarsphenamine is, how high over the M. E. D. may we go without danger of reaching the minimum lethal dose?

The average M. E. D. for arsphenamine is 3.1 gm. per kilo body weight; for neoarsphenamine, 3.4. These two doses are approximately the same. The M. L. D. on the contrary is 53.2 for arsphenamine and 96.5 for neoarsphenamine. Once again we see a wider margin of safety in the latter drug. Voegtlin and Smith designate as the "therapeutic ratio," the ratio $\frac{\text{M. L. D.}}{\text{M. E. D.}}$ For arsphenamine this is 17.2, and for neoarsphenamine 28.4. The higher the ratio the less danger is there in administering the drug in amounts greater than the M. E. D.

These experimental observations coincide with clinical knowledge that toxic reactions occur less frequently after large doses of neoarsphenamine than after corresponding doses of arsphenamine.

Schamberg, Kolmer and Raiziss[9] have made a study of the relative toxicity of the two drugs. They found that for rats and mice neoarsphenamine is about

2.4 times less toxic than is arsphenamine. They conclude that, as far as is indicated by intravenous experiments on rats, the usual dose of 0.6 gm. of arsphenamine is about 12 times less than the highest tolerated dose, while 0.9 gm. of neoarsphenamine is 19 times less than the highest tolerated dose for the latter drug. Here again the conclusion is that larger amounts of neoarsphenamine are of less danger than are proportionate amounts of arsphenamine.

It is well known that neoarsphenamine may be given with safety at shorter intervals than arsphenamine. Will it not be true then, that if the former drug is administered in slightly larger doses and somewhat more frequently, say 0.9 gm. thrice weekly, equally good therapeutic results will be obtained? This raises a question as to whether the difference in therapeutic results is due merely to a difference in dosage or whether it depends on a chemical difference between the two drugs.

Swift[10] studied the rate of absorption of arsenic from the tissues after the intramuscular injection of both arsphenamine and neoarsphenamine. His work was done on rabbits. At the end of one week more than twice as much of the neoarsphenamine had been absorbed as of the arsphenamine. Lockemann[11] found that neoarsphenamine was excreted more rapidly. The latter drug is apparently absorbed more rapidly after local injection and is eliminated more rapidly from the body after absorption. Voegtlin and Smith suggest that arsphenamine is probably retained longer in the body than is neoarsphenamine because it is precipitated at the hydroxyl-ion concentration of the blood and therefore must be temporarily fixed in the tissues in a nonreactive state. This insoluble form is probably slowly oxidized in the body to the parasiticidal oxide and is gradually distributed as such through the blood stream. Neoarsphenamine on the contrary is not precipitated at the hydroxyl-ion concentration of the blood and its oxidation and elimination therefore proceed more rapidly. It is probable that this hypothesis represents the actual state, but it does not necessarily follow that this is the entire or the correct explanation for the therapeutic differences.

It is a curious fact that, experimentally, neoarsphenamine is usually more efficacious than is arsphenamine. But the laboratory observations are usually carried out on trypanosome infections or spirillosis, in which the parasite is predominantly in the blood stream. Here the relative rates of excretion are of less importance than in human syphilis. In the latter case it is important to reach the deep-seated foci, and this requires time. Too rapid excretion would prevent the drug from reaching deep-seated foci in the tissues.

Brown and Pearce have, however, observed that in rabbit syphilis neoarsphenamine is almost always more efficacious than is arsphenamine.

Schamberg[12] concluded from comparative studies that the addition of the formaldehyde sulphoxylate group found in the neoarsphenamine, may lessen the affinity of this drug for the protoplasm of the parasite, but at the same time lessening the affinity for the body proteins to an even greater extent. This hypothesis would explain both the diminished therapeutic effect and also the decidedly lessened toxicity. Another phenomenon bearing on relative toxicity is the observation by Schamberg that arsphenamine in practically all concentrations hemolyzes red blood cells *in vitro,* while neoarsphenamine does not do so in

any concentration clinically employed. Schamberg has concluded that arsphenamine is more active therapeutically but that this advantage is balanced by the much higher tolerated dosage of neoarsphenamine and by the fact that the latter is less likely to cause biochemical disturbances in the blood and the tissues.

A point of great practical interest is that recently made by Roth[13] who has found that the shaking of alkalinized aqueous solutions of arsphenamine and neoarsphenamine in the air for 60 or even 30 seconds, increases greatly the toxicity, probably by oxidation. This knowledge should lead to caution in the preparation of solutions for use in treatment.

The preceding review indicates fairly clearly the unsettled status of arsphenamine and neoarsphenamine dosage. At present there is sufficient evidence to justify those who prefer to use the simpler technic of neoarsphenamine therapy, but only on the condition that sufficiently large and frequent doses be administered. With either method, mercury and the iodides should not be neglected. Therapeutic results will be the ultimate criterion as to the relative advantages of arsphenamine and neoarsphenamine. We will await with interest further reports from the clinic and from the laboratory not only because of the practical interest but also because of the present inconclusive literature on the subject.

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—*W. T. V.*

Hypersensitiveness to Foods as Cause for Abdominal Pain

SEVERE abdominal pain, says Duke, is a symptom which is never taken lightly by a careful physician. It often indicates a severe illness; in fact it often indicates an emergency. But, says Duke, there are cases in which abdominal pain may simulate that arising in serious abdominal disease, but which is the result of hypersensitiveness to food. It has been known for a number of years that hypersensitiveness to foods may give rise to bronchial asthma and a condition which simulates hay fever; to urticaria, angioneurotic edema, purpura, eczema, and other dermatoses; to dyspepsia, gastrointestinal upsets associated with vomiting, diarrhea, griping pains in the abdomen, and mucous colitis; to an interesting syndrome of symptoms known as Henoch's purpura. With the latter conditions a patient may have severe abdominal pain.

But abnormal pain sometimes occurs alone and is the sole striking symptom of hypersensitiveness to a food. In such cases the symptoms may be misleading in diagnosis. It is such cases that Duke discusses. He has observed a number of patients who have been sensitive to one or more of the following foods: egg white, egg yolk, shad roe, lactalbumin, casein, beef, pork, honey, strawberries, lettuce, almonds, beans, onions, cabbage, rice, potatoes, tomatoes, paprika and pimento, and who have invariably had an attack of abdominal pain whenever they have eaten the foods to which they were sensitive. In the majority of cases pain appeared soon after ingestion of the food and lasted for three to six hours. In several cases it did not appear until several hours later and then it lasted much longer. In the majority of cases pain was associated with nausea and vomiting, less frequently with indigestion, bloating, diarrhea and mucous stools, and much less frequently with hives, angioneurotic edema and purpura.

The pain complained of was evidently the result of a reaction caused by contact between the gastrointestinal mucosa and the food to which it was sensitive, and this contact gave rise to gastrointestinal symptoms in much the same way that contact of the mucous membrane of the respiratory tract with a pollen to which it is sensitive gives rise to symptoms of hay fever or asthma. In other words the pain and other gastrointestinal symptoms seem fundamentally analogous in pathogenesis to the symptoms of hay fever and asthma. An attack of asthma can be brought on in a sensitive individual by a subcutaneous injection of the pollen to which he is sensitive. Analogous to this several patients had gastrointestinal pain after subcutaneous injections of an extract of the food to which they were sensitive.

Perhaps the most interesting part of Duke's communication is that in which he calls attention to the fact that nearly 50 per cent of his patients who showed food hypersensitiveness had demonstrable pathologic lesions in the alimentary tract or its appendages. These lesions were recurrent appendicitis, gall stones, duodenal ulcer, dense adhesions and extreme ptosis. So, it is suggested, that while allergy is primarily dependent upon an inherited constitution which renders a person susceptible of becoming hypersensitive to certain alien substances, it is also possible that an abnormality in the alimentary tract may be a contributory factor in the etiology. It may even be the starting point of an inheritance if one can accept inheritance of acquired characteristics.

The diagnosis in these cases was founded upon careful observation of the diet and the physical reactions of the patients; they were confirmed by cutaneous tests.

Duke's paper appears in the *Archives of Internal Medicine,* 1921, xxviii, 151. The most recent discussion of the subject of inheritance of acquired characteristics is contained in Bernard Shaw's "Back to Methusela," Brentano, 1921.

—*P. G. W.*

The National Board of Medical Examiners

THE National Board of Medical Examiners has just completed the first five years' work and with it the trial period of its usefulness. The principle which this Board has stood for, namely, the establishment of a thorough test of fitness to practice medicine which might safely be accepted throughout this country and abroad, has been widely accepted. Since this Board was organized by Dr. W. L. Rodman, in 1915, eleven examinations have been held. These examinations have been conducted on the plan of holding at one sitting, a written, practical and clinical test for candidates with certain qualifications, namely a four-year high-school course, two years of college work, including one year of Physics, Chemistry, and Biology, graduation from a Class *A* Medical School and one year's internship in an acceptable hospital. These examinations have covered all the subjects of the medical school curriculum and have been conducted by members of the Board with members of the profession resident in the place of examination appointed to help them. Such examinations have been held in Washington, Philadelphia, New York City, Boston, Chicago, St. Louis, Rochester (Minnesota) and Minneapolis. During the war a combined examination was held at Fort Oglethorpe and Fort Riley. There have been 325 candidates examined, of whom 269 have passed and been granted certificates.

Starting with the endorsement of the Council on Medical Education of the American Medical Association, America Medical College Association and various sectional Medical Societies, the recognition of the Army, Navy and Public Health Service Medical Corps of the United States and certain State Boards of Medical Examiners, the certificate is now recognized. Also by twenty states as follows: Alabama, Arizona, Colorado, Delaware, Florida, Georgia, Idaho, Iowa, Kentucky, Maryland, Minnesota, Nebraska, New Hampshire, New Jersey, North Carolina, North Dakota, Pennsylvania, Rhode Island, Vermont and Virginia, the Conjoint Board of England, the Triple Qualification Board of Scotland, the American College of Surgeons and the Mayo Foundation of the University of Minnesota.

There has been such a widespread demand for an opportunity to secure this Certificate by examination, that the Board has now adopted and will put into effect at once, the following plan: Part I, to consist of a written examination in the six fundamental medical sciences: Anatomy, including histology and embryology; Physiology; Physiologic Chemistry; General Pathology; Bacteriology; Materia Medica and Pharmacology. Part II, to consist of a written examination in the four following subjects: Medicine, including pediatrics, neuropsychiatry, and therapeutics; Surgery, including applied anatomy, surgical pathology and surgical specialties; Obstetrics and Gynecology; Public Health, including hygiene and medical jurisprudence. Part III, to consist of a practical examination in each of the following four subjects; Clinical Medicine, including medical pathology, applied physiology, clinical chemistry, clinical microscopy and dermatology; Clinical Surgery, including applied anatomy, surgical pathology, operative surgery, and the surgical specialties of the diseases of the eye, ear, nose and throat; Obstetrics and Gynecology; Public Health including sanitary bacteriology and the communicable diseases.

Parts I and II will be conducted as written examinations in Class A Medical Schools and Part III will be entirely practical and clinical. In order to facilitate the carrying out of Part III, subsidiary boards will be appointed in the following cities, Boston, New York, Philadelphia, Minneapolis, Iowa City, San Francisco, Denver, New Orleans, Baltimore, Galveston, Cleveland, St. Louis, Chicago, Washington, D. C., and Nashville and these boards will function under the direction of the National Board. The fee of $25.00 for the first part, $25.00 for the second part and $50.00 for the third part will be charged. In order to help the Board the Carnegie Foundation has appropriated $100,000.00 over a period of five years.

At the Annual Meeting held June 13th, of this year in Boston, the following officers were elected, M. W. Ireland, Surgeon General, President; J. S. Rodman, M.D., Secretary-Treasurer, E. S. Elwood, Managing Director.

Mr. Elwood will personally visit all Class A Schools during the college year to further explain the examination, etc., to those interested. Further information may be had from the Secretary-Treasurer, Medical Arts Building, Philadelphia.